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ROBERT WILLIAM HEGNER
1880–1942

By the death of Robert Hegner on March 11, 1942, parasitology in the United States has lost another of its important leaders. For almost a quarter of a century he had carried on extensive researches on the parasitic protozoa and had been the director of an active group of students and investigators in this field. He was one of the founders of the American Society of Parasitologists, having been a member of the original committee that drew up its constitution and called the first meeting in Washington in 1925. When the Society took over the JOURNAL OF PARASITOLOGY in 1932 he became the representative of protozoology on the first editorial committee. He was vice-president of the Society in 1928 and its president in 1936. His relations with foreign workers in parasitology have also been unusually close not only from the exchange of publications but also from personal contacts during his extensive travels. To his numerous students he was a close friend and advisor, and his charming personality and rare sense of humor made him a favorite with his colleagues. Parasitologists will long remember the delightfully informal way in which he presided at the meetings of the Society at Atlantic City in 1936.* Those of us who were with him during the last months of his life will never forget the brave fight that he made, when in spite of increasing pain and weakness he refused to give up and continued his lectures and the direction of his department until a few weeks before his death.

Dr. Hegner was born in Decorah, Iowa, on February 15, 1880. He was graduated from Lyons' Institute in Chicago and then continued his education in the University of Chicago where he received the A.B. degree in 1903 and the M.S. degree in 1904. During this period he acted as assistant in the Department of Zoology, spent one summer on a scientific expedition in Mexico, and several summers at the Marine Biological Laboratory at Woods Hole. Later, he went to the University of Wisconsin where he received the Ph.D. degree in 1908, the title of his dissertation being "The Origin and Early History of the Germ Cells in

* Dr. Hegner's portrait, and presidential address given at Atlantic City, entitled "Parasite Reactions to Host Modifications," were published in this JOURNAL, February, 1937 (**23**: 1).

Some Chrysomelid Beetles." During the years 1908 to 1917, he was an instructor and then assistant professor of zoology at the University of Michigan. At Michigan, he developed further his interest in the germ cell cycle of insects, publishing a series of about 20 papers on this subject and a book, "The Germ Cell Cycle in Animals," which appeared in 1914. During this period, also, he published his "Introduction to Zoology," 1910, and the first edition of his "College Zoology," 1912, which has continued as a leading text book up to the present time. In fact, the fifth edition of the College Zoology was finished just before his death, and will be published this summer.

During 1917 and 1918, he held a Johnston Scholarship in the Department of Zoology of the Johns Hopkins University. Here he carried out his first researches on the protozoa and published a series of five papers on variation and heredity in the free-living species, *Arcella dentata*. While in Baltimore, he received an offer of a position in the newly organized School of Hygiene and Public Health of the Johns Hopkins University. Seeing clearly the possibilities of the field of parasitology in relation to public health, he joined the staff of this institution in 1918. Here he organized the work in medical zoology and developed research and teaching in protozoology. He served this institution for more than two decades, devoting himself to the study of the parasitic protozoa, but fostering, also, active work in the whole field of parasitology. His own researches covered all phases of his subject, and his publications during this period were very numerous, totaling almost 150 titles. In addition, he stimulated research activity in those around him. Forty students received the doctorate under his direction and a number of other investigators studied in his laboratory. After a few years, his department became one of the most active centers of research in parasitology in the world.

Dr. Hegner was a great traveller, and his researches on the protozoan diseases of man took him frequently into the field. He directed several expeditions in tropical America, spent a year in the Philippine Islands, and in 1938 worked for five months at the Institute of Public Health in Mexico City. In addition, he travelled widely in Europe, the Orient, and South America. He was a member of many scientific societies in this country and abroad, and was on the editiorial boards of a number of journals of zoology, parasitology, and tropical medicine.

Dr. Hegner's personal contributions to parasitology were many and varied. He was the author of the following books in this field: (1) Diagnosis of Protozoa and Worms Parasitic in Man (with W. W. Cort), 1921; (2) Outlines of Medical Zoology (with W. W. Cort and F. M. Root), 1921; (3) Human Protozoology (with W. H. Taliaferro), 1924; (4) Host-Parasite Relations between Man and His Intestinal Protozoa, 1927; (5) Problems and Methods of Research in Protozoology (with

J. Andrews), 1930; (6) Parasitology, 2nd ed. (with F. M. Root, D. L. Augustine, and C. G. Huff), 1938; and (7) Big Fleas Have Little Fleas, 1938.

In research, while centering his attention on the human protozoa, he also constantly developed the zoological aspects of the subject. It would take too long to try to analyze in detail his research contributions. A number of his papers dealt with the systematics and morphology of the protozoa of man and other animals. Still others gave the results of surveys of human intestinal protozoa in countries which he visited. His main interest, however, was in the study by experimental methods of the factors influencing the transmission of parasitic protozoa and of the relations of the host to its parasites. In this connection he introduced the term "host-parasite relations," which has been widely used. One line of research on host-parasite relations which he started rather early and came back to from time to time was the relation of the diet of the host to its infection with intestinal protozoa. Of most interest, perhaps, in this connection was the finding that a carnivorous diet in mammals is unfavorable for the intestinal flagellates. Another series of his investigations was concerned with the biology of intestinal protozoa, particularly *Trichomonas* and other flagellates, as studied in vitro and in laboratory animals, particularly the rat and newly hatched chicks. Also, on one of his trips to Panama he had the opportunity of studying experimental amebiasis in monkeys and made significant contributions to our knowledge of the histopathology of this disease.

He had early recognized the importance of experimental studies on malaria and using various species and strains of bird malaria, carried out with the aid of a number of his students studies on all phases of the malarial problem. During the last six years, these studies completely dominated his interest and absorbed the whole research efforts of his department. Of the numerous results that have come out of this program on bird malaria, perhaps, the most important have been those that dealt with the significance of acquired immunity and with the relation of the susceptibility of young red blood cells to the course of the infections. In his last paper, published in September, 1941, he reported the successful treatment of bird malaria with a new drug, which is now being tried on human malaria.

Dr. Hegner's personal accomplishments in research were supplemented by the investigations of his numerous students and the visiting investigators in his laboratories. A large proportion of his students are still engaged in research in protozoology, and some already hold positions of leadership. Thus, his influence will long continue. He lived a very full and rich life, and left behind him an unusually large body of accomplishments.—W. W. Cort, *School of Hygiene and Public Health, Johns Hopkins University.*

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LIFE HISTORY STUDIES ON TWO TREMATODES OF THE SUBFAMILY NOTOCOTYLINAE*

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During the summer of 1939 several species of notocotylid cercariae were isolated from naturally infected snails collected from the Douglas Lake region, Michigan. Cysts formed from cercariae of two of these species were fed to a wide variety of different animals and adults were obtained in suitable hosts. In 1940, intermediate hosts were infected with eggs obtained from the adults of these two species and the early larval stages were followed in the snail intermediate hosts.

A review of the literature showed accounts of about ten experiments in which cercariae and adults were connected in notocotylid life cycles. In four cases the adults were identified as *Notocotylus attenuatus* Rud. [Joyeux (1922), Mathias (1930), L. and U. Szidat (1933), and Yamaguti (1938)]. The others dealt with forms that were identified as six different species [Harper (1929), L. and U. Szidat (1933), U. Szidat (1935), Luttermoser (1935), and Yamaguti (1938)]. In only one case was the snail intermediate host experimentally infected (Joyeux, 1922). Joyeux, therefore, gave the only description of a notocotylid mother sporocyst. Only in a few cases have two generations of rediae been reported for the larval stages in this group.

The present communication deals with life history studies on two species of notocotylid trematodes parasitic in mammals and birds. The first, *Quinqueserialis quinqueserialis* (Barker and Laughlin, 1911), has been reported previously only in the adult stage. Its intermediate stages are found in *Gyraulus parvus* (Say). The second, *Notocotylus stagnicolae* n. sp., is previously undescribed in any stage. Its intermediate stages are found in *Stagnicola emarginata angulata* (Sowerby) and *S. e. canadensis* (Sowerby).

MATERIALS AND METHODS

Natural infections of larval stages were obtained in snails collected from Douglas Lake, Walloon Lake, and Burt Lake, Michigan. Mature cercariae were studied alive and also from permanent whole mounts

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stained in Ehrlich's acid hematoxylin. Some cercariae were fixed in hot 10 per cent formalin for measuring. Metacercariae were studied alive and as permanent whole mounts stained in paracarmine. Lettuce-lined stender dishes were used in obtaining cysts for feeding experiments.

Of the experimental final hosts, albino and hooded rats, ducks, and part of the chickens were raised in the laboratory. The rest of the chickens were purchased from nearby farms and the meadow mice were trapped. The adult worms were studied alive or fixed in Gilson's fluid heated to about 65° C. Sections were made from material fixed in Bouin's solution. Freshly shed eggs were fixed by dropping into hot fixing solutions.

Laboratory raised snails, *S. e. angulata,* were used for the infection experiments with *N. stagnicolae.* Small specimens of *G. parvus* with a low incidence of infection with *Q. quinqueserialis,* were used for the infection experiments with that species. It was therefore necessary to take special precautions so that natural infections would not be mistaken for experimental infections in these snails. A week after exposure to freshly shed eggs, snails were first examined with a dissecting microscope for evidences of infection.

In connection with the study of ventral glands in adult worms a new method was devised by which a permanent impression of fixed worms could be made on the film side of lantern slides. The unused lantern slide is cut to the size of microscope slides and placed in hypo for 20 minutes. Then the strips are washed in water and allowed to dry until the gelatin is of rubber-like consistency. The ventral side of the worm is flattened on this film for several minutes with the pressure of a lead weight on top of a strip of heavy glass. The worm is shortly peeled off and an impression is left permanently on the film which shows clearly the position and number of ventral glands.

THE LIFE CYCLE OF *Quinqueserialis quinqueserialis* (BARKER AND LAUGHLIN)

The adult stage of *Q. quinqueserialis* is a parasite of the cecum of muskrats and meadow mice. Eggs deposited with the cecal material gradually pass out with the feces. A group of experimental snails, which was examined at intervals for 26 days after exposure to the eggs, gave information on the course of development in the intermediate host (Table 1).

The Eggs and Infection of Intermediate Hosts.—Adult worms, raised experimentally in the meadow mouse, were placed in stender dishes containing saline. A short time later filamentous eggs were extruded from the genital pore in the form of ropy masses. The shell of the egg is light brown, oval, operculate, and has polar filaments. Twenty eggs fixed in

TABLE 1.—*Sequence of stages of* Q. quinqueserialis *in the intermediate host,* Gyraulus parvus, *exposed to eggs on July 27, 1940*

Age of infection in days*	Number of stages found	Stage of development	Position in snail
9	6	Immature mother sporocysts	Mantle
10	2	" " "	"
11	32	" " "	"
13	8	Mature mother sporocysts	"
14	2	" " "	"
	2	Mother rediae	Along intestine
16	3	Mature mother sporocysts	Mantle and along intestine
17	2	" " "	Mantle
	38	Mother rediae	Along intestine
18	1	Mature mother sporocyst	Found loose
	1	Mother redia	Along intestine
19	7	Mother rediae with d. rediae	Along intestine and in digestive gland
20	1	Mother sporocyst	Mantle
	40	Mother rediae (estimated)	Along intestine and in digestive gland
	4	Daughter rediae	In digestive gland
21	1	Mother sporocyst	Mantle
22	47	Rediae (mother and daughter)	Along intestine and in digestive gland
23	32	Rediae (mother and daughter)	In digestive gland
	14	Immature cercariae	" " "
24	43	Rediae (mother and daughter)	Along intestine and in digestive gland
	9	Immature cercariae	In digestive gland
25	88	Rediae (mother and daughter)	" " "
	21	Immature cercariae	" " "
26	46	Rediae (mother and daughter)	" " "
	11	Immature cercariae	" " "
		Mature cercariae	" " "

* One snail was examined on each of these days.

hot Gilson's had lengths varying from 0.018 to 0.021 mm and widths from 0.011 to 0.014 mm. The lengths of the polar filaments varied from 0.268 to 0.436 mm. In spite of the opacity of the egg shell which made observations of the contents difficult, they could be seen to contain miracidia.

On July 27, 1940, several lots of small specimens of *G. parvus* were exposed to eggs of *Q. quinqueserialis.* A control series of a dozen snails from the same collection was not exposed to eggs. The following descriptions except for older cercariae and metacercariae were made from this experiment in which 25 out of 28 snails were infected with young larval stages. The other three snails were found to have mature natural infections of this species and unfortunately were not examined any further for young larval infections. Controls had a similar percentage of mature natural infections; the rest were negative. Some laboratory raised *Physa gyrina* were also exposed to eggs of *Q. quinqueserialis,* but examinations two weeks later revealed no evidence of infection.

The Mother Sporocyst (Figs. 1, 2).—The youngest mother sporocysts recognized were found imbedded within the mantle lining as early as 9 days after exposure to infection. The sporocyst at this stage is sac-like, oval or round, and measures between 0.023 and 0.026 mm in diameter. The wall is lined with a layer of epithelial cells. A mass of cells is loosely attached to the inside wall. Ten days after exposure more of these oval sacs were found containing germinal masses of different sizes. In later examinations up to 21 days after infection sporocysts of increasing size

were found in which the rediae could be distinguished (Table 1). Some were firmly imbedded in the mantle; others were loosely attached along the intestine, and a few were found loose in the dish when the snail was opened. The largest sporocyst found measured 0.243 mm in diameter. The number of sporocysts found in a single snail varied, the highest being 32. The number of rediae inside the sporocyst is always 4. No birth pore was observed in the sporocyst wall and apparently the mother rediae are able to break through the wall at any point.

The Mother Redia (Fig. 3).—As early as 14 days after exposure small, almost colorless mother rediae were found free along the intestine; the smallest was 0.125 mm long and 0.053 mm wide. After the mother redia inside the sporocyst has developed a pharynx, a narrow hyaline lumen with dilations extends from the pharynx into the body cavity to form the intestine. This later enlarges to form a large sac-like intestine filled with dark yellowish granules and occupying a good deal of the body cavity of the redia. In a large redia the pharynx measured 0.038 by 0.034 mm. The mouth is terminal and capable of considerable protrusion and contraction. Seven mother rediae containing daughter rediae were found in the digestive gland of a snail 19 days after exposure. One of these rediae had 5 daughter rediae and 17 germ balls. The largest mother redia seen measured 0.70 by 0.20 mm. It was impossible in the experimental snails to differentiate immature mother and daughter rediae free in the tissues.

The Daughter Redia (Fig. 4).—Daughter rediae were observed free in that part of the digestive gland nearest to the stomach 20 days after infection. At the time of the escape from the mother redia a daughter redia measured 0.109 by 0.046 mm. Older daughter rediae are yellowish elongate sacs, narrow at the anterior end and wider towards the posterior end. They are like mother rediae and can only be distinguished from them by the presence of developing cercariae. The smallest daughter redia containing embryos that could be recognized as developing cercariae measured 0.41 by 0.16 mm; the largest 1.31 by 0.23 mm. Most mature daughter rediae contain a few well developed cercariae and about a dozen germ balls in various stages of development. In many cases eye-spots in these cercariae are well developed and are visible through the walls of the rediae.

The Cercaria (Figs. 5–8).—Cercariae, almost mature, emerge from the birth pore of the rediae and migrate into the digestive gland where a short period of maturation occurs. Maturing cercariae, 23 days old, exhibited little motion and it was not until 3 days later that actively swimming cercariae were observed. Under favorable conditions of light and temperature fully developed cercariae may be seen emerging from the snails between the hours of 9 and 11 A.M. The body is usually ovoid but

almost finger-like when fully extended. The tail when extended is almost twice as long as the body and capable of considerable extension and contraction. Ten fixed specimens measured 0.23 to 0.28 mm in length by 0.13 to 0.17 mm in width. The tail varied in length between 0.39 and 0.52 mm. The whole body is opaque and packed with cystogenous cells. The excretory system consists of two trunks joined anteriorly and discharging posteriorly into the sides of the excretory bladder. The excretory tubes join to form a finger-like diverticulum below or to the side of the median eye-spot. A large number of fine granular excretory concretions, unequally distributed, are found in these tubes.

The Metacercaria (Fig. 9).—After the cercariae emerge from the snail they readily encyst on vegetation or on the surfaces of the containing vessel. The process of encystment is completed in 3 to 5 minutes. Ten cysts scraped from the surface of a stender dish on which they had formed 20 minutes previously varied in diameter from 0.16 to 0.18 mm. In side view they are hemispherical, the attached surface being flat. The metacercaria is infective soon after encystment and may remain so for at least 3 weeks if kept refrigerated.

Infection of Definitive Hosts.—Cysts, from naturally infected snails, formed on lettuce were fed to 4 ducks, 1 pigeon, 1 chicken, 1 rat, and 9 meadow mice but only the latter were successfully infected. The mice used in these experiments were kept in the laboratory for some time before they were fed cysts.

The first 2 mice that were fed cysts died three and eight days after infection. Both harbored large numbers of immature worms. Later 2 more mice were fed about 40 cysts apiece and after 10 days their fecal pellets were examined daily. In one filamentous eggs were detected 16 days after feeding the cysts. This mouse was sacrificed 23 days after feeding and found to have 10 adults in the cecum and 4 in the ileum. The other mouse was found to harbor 39 adult worms when it was autopsied 29 days after infection. These specimens appeared to be fully grown as eggs were found in the feces for 12 days prior to sacrificing the host. Five other mice were successfully infected and worms of different ages recovered. Specimens of the same species were also obtained from naturally infected muskrats at Douglas Lake, Michigan and Carlisle, Pennsylvania. Three specimens from the U. S. Nat. Mus. Helm. Coll., Nos. 28185, 40961, and 40981 taken from the cecum of muskrats and used by Harwood (1939) in his description of *Q. quinqueserialis,* were also available for comparison. No opportunity was presented to study the original material described by Barker and Laughlin as *Notocotylus quinqueserialis.*

Adult (Figs. 10, 12).—Because the earlier descriptions were neither made from experimental material nor from worms of known age additional data on this species is given with measurements of 20 whole mounts from the 29-day-infection in the meadow mouse.

Description: Medium sized, oval, muscular flukes, 2.91 ± .29 (2.53–3.51) mm long by 0.93 ± .11 (0.86–1.09) mm wide; anterior end more or less pointed, posterior end rounded; cuticle beset with minute spines on anterior half of ventral surface; oral sucker large, ventral, 0.34 ± .04 (0.21–0.43) mm in diameter; esophagus very short, bifurcating almost immediately; ceca irregular tubes descending to some distance below posterior median margin of testes with many dilations and indentations; genital pore ventral, posterior to intestinal bifurcation; five rows of ventral glands, number in lateral rows 16 to 19 with a mean of 17, in middle row 14 to 16 with a mean of 15; testes lobed, 0.48 ± .05 (0.41–0.60) mm long by 0.27 ± .03 (0.21–0.35) mm wide; cirrus eversible, echinate; ovary lobed, 0.28 ± .03 (0.22–0.34) mm long by 0.22 ± .02 (0.17–0.25) mm wide; vitellaria lateral, with about 12 to 17 groups of follicles on each side; position of vitellaria (length of body divided by forward extent of follicles) 1.9 to 2.2 with a mean of 2.1; uterus continues forward from the oötype in numerous transverse coils some of which extend beyond the ceca; uterine loops between vitellaria and cirrus sac on side of metraterm 4 to 7 with a mean of 6; seminal receptacle absent but replaced by a seminis receptaculum uterinum; excretory bladder receives 2 branched excretory tubules joined anteriorly below the oral sucker.

Only three described species resemble my material which has a large oral sucker and 5 rows of ventral glands. They are *Q. quinqueserialis* (=*Notocotylus quinqueserialis* Barker and Laughlin, 1911) from the muskrat and also from the meadow mouse (Harrah, 1922), *Notocotylus hassalli* McIntosh and McIntosh, 1934, from *Microtus pennsylvanicus* and from *Zapus hudsonius* (Erickson, 1938), and *N. wolgaensis* Skvortsov, 1935, from *Arvicola terrestris.* Comparisons indicate that my specimens were not *N. hassalli* because they have a smaller number and smaller clusters of vitelline follicles. Also they are not *N. wolgaensis* because it has the same number of ventral glands in each row. They were found to belong to *Q. quinqueserialis.*

The above description of *Q. quinqueserialis* agrees with the original except in a few points. The original stated that there are from 16 to 18 ventral glands in each row but in more than 80 specimens examined I have found the most common number of glands in the five rows to be 15 for the median and 17 for all the other rows. The next most common combination was 15 for the middle row and 18 for all the other rows. The highest total number counted in one specimen was 92 in which the middle row had 16 and all the other rows had 19. Large worms had about the same number of glands as smaller specimens. The original description also stated that the excretory tubules end blindly whereas they are found to join anteriorly below the oral sucker. The variations in body measurements are also greater in the original than in my material which was all of the same age. These differences are minor and can be explained as due to the small number and differently fixed specimens used for description. My material agrees with the redescription of *Q. quinqueserialis* given by Harwood (1939).

THE LIFE CYCLE OF *Notocotylus stagnicolae* N. SP.

The second species, *N. stagnicolae* n. sp., has a life history similar to

that of *Q. quinqueserialis.* In this case, however, the intermediate hosts are varieties of *Stagnicola emarginata* and the adults develop more readily in birds than in mammals.

The Eggs and Infection of Intermediate Hosts.—The eggs of *N. stagnicolae* obtained from an experimental chicken are slightly larger than those of *Q. quinqueserialis.* The polar filaments are also longer (Fig. 23). Twenty fixed specimens measured 0.021 to 0.025 mm in length by 0.014 to 0.017 mm in width. The filaments varied in length from 0.50 to 0.62 mm.

On August 5, 1940, eggs from 84 worms, taken from an experimental chicken, were placed with about 200 laboratory-raised juveniles of *S. e. angulata.* A week after exposure some of these snails were examined. After this, examinations were made daily with few exceptions for a month, and then at longer intervals until the termination of the experiment 8 months after infection. Every one of 97 snails examined was infected with some larval stage of this species. *G. parvus* and *P. gyrina,* exposed to eggs of *N. stagnicolae,* were found uninfected 23 days later.

No eggs were seen to hatch but infection probably occurred by penetration of the miracidia. The descriptions of all the larval stages except the daughter rediae and cercariae, which were studied from natural infections in *S. e. canadensis* and *S. e. angulata,* were made from material found in the examinations of these experimental snails. After 18 days the weather became much cooler and the lower temperatures in the laboratory probably retarded development considerably. Therefore, the time of appearance of the various stages and the sequence of development cannot be considered as normal in this experiment.

The Mother Sporocyst (Figs. 13–16).—The first mother sporocysts were found 7 days after infection. They were small spherical sacs about 0.035 mm in diameter, attached along the intestine by very thin membranous tissue. At this stage I could not distinguish any germ cells or developing embryos inside the sporocysts. The earliest embryo of a mother redia which consisted of about 8 cells was found 9 days after infection suspended in the cavity of a sporocyst 0.053 mm in diameter. In another sporocyst in the same snail a single developing redia was observed in which a pharynx could be made out. Many sporocysts each containing a single developing mother redia were found about 2 weeks after infection (Figs. 13–15). Eighteen days after exposure a well developed sporocyst (0.239 mm in diameter) was found containing a mother redia (Fig. 16) similar to those found free in the tissues along the intestine or in the digestive gland of the same snail. Sporocysts always contained only one redia and were found in the snails up to 33 days after infection. The heaviest reported sporocyst infection was 23, distributed throughout the mantle and along the intestine. Several sporocysts in other snails were found in the digestive gland.

The Mother Redia (Fig. 17).—As early as 14 days after infection and in most examinations for 8 months afterwards, mother rediae were found in the tissues of the experimental snails. At first they were located along the intestine but later they were all found in the digestive gland. The smallest free mother redia measured was 0.17 mm long by 0.08 mm wide; the largest 1.33 mm long by 0.19 mm wide. The number of free mother rediae in any one snail varied between one and 22 although one snail had 11 mother rediae and 15 mother sporocysts each containing one redia making a possible total of 27.

Mother rediae first showed food particles in the intestines 22 days after infection of the snails. The number and size of the food particles and the amount of pigmentation in the body wall increased as development progressed. In infections of 3 months or longer the mother rediae could be distinguished rather easily from the large number of daughter rediae by their larger size and deeper pigmentation. One large mother redia contained about 27 germ balls and another 15 daughter rediae and about 12 germ balls. Keeping the snails in the laboratory during the winter and feeding them bits of lettuce apparently produced conditions which brought about degeneration of daughter rediae. Besides an actively moving daughter redia one old mother redia contained the anterior ends of 2 daughters each consisting of a pharynx and a small portion of the body posterior to it. Why one of the rediae was apparently normal while 2 others in the same mother were degenerating is difficult to explain unless we suppose that the active redia had just matured and would degenerate later.

The Daughter Redia (Fig. 18).—Daughter rediae with pharynx and narrow intestinal lumen were first seen in the mother rediae of experimental snails 24 days after exposure. One of the larger developing daughter redia within the mother measured 0.37 mm in length by 0.17 mm in width. One of the first daughter rediae found free in the digestive gland of a snail 27 days after infection measured 0.389 by 0.194 mm. The number of daughter rediae in the experimental snails gradually increased. In one 45-day-infection there were 38; in 51 days about 50; in 75 days about 90; in 100 days about 100; in 195 days about 500. My estimate is that each mother redia produces at least 25 or 30 daughters. The large numbers of rediae found in the experimental snails was due to multiple infection.

Twenty-five mature fixed specimens of daughter rediae obtained from a natural infection in *S. e. angulata* varied in length from 0.70 to 1.15 mm and in width from 0.17 to 0.27 mm. The pharynx varied from 0.059 to 0.075 mm in diameter. The esophagus was short and measured between 0.011 and 0.041 mm in length. The intestine of mature specimens is filled with particles of various sizes and extends into the posterior third of the

body. The number of developing cercariae far enough advanced to show eye-spots is usually 3. The number of germ balls varies from 8 to 16. In several rediae the germ balls appeared to be concentrated in a funnel-shaped structure near the posterior end (Fig. 18). The most fully developed cercariae inside the rediae contained pigment material in the anterior end and an outline of the excretory trunks devoid of granules. The color of mature rediae is usually yellowish or brown.

The Cercaria (Fig. 19).—Notocotylid cercariae emerging from one specimen of *S. e. canadensis* collected from Walloon Lake, Michigan, were first suspected of being new for the region because they escaped late in the morning or shortly after noon. Their encystment was also delayed; in some cercariae the interval between emergence and cyst formation was as long as 20 minutes. Other observations on the course of the excretory tubules and measurements of body, tail, and cyst made it certain that this was a new species. Eleven additional infections of this type of cercaria were found in specimens of *S. e. angulata* collected in Burt Lake. Metacercariae from both collections were fed to experimental animals and identical adults recovered.

Eggs from some of these adults served as a source of infection for the experimental snails. Generally the digestive glands of these snails contained very few cercariae in spite of the length of the infection and the large numbers of rediae present. Five free swimming cercariae were recovered from one snail 75 days after exposure to infection. A normal daily cercarial output from a naturally infected snail is usually over 50.

The cercariae of *N. stagnicolae* have 3 eye-spots when mature but in undeveloped cercariae the pigment is diffuse around the anterior end, with clumps in the area of the 2 lateral eye-spots. The bodies of 25 free swimming specimens fixed in Gilson's measured 0.32 to 0.57 mm in length by 0.08 to 0.21 mm in width. The tails varied in length between 0.62 and 0.94 mm or about twice as long as the body. The body is not as opaque as that of the cercaria of *Q. quinqueserialis*. The main parts of the excretory system consists of 2 trunks joined anteriorly below the median eye-spot and discharging posteriorly into the anterior sides of the excretory bladder.

The Metacercaria (Fig. 20).—Encystment sometimes occurred on the shell of the snail from which the cercariae were emerging although other surfaces are the usual sites for cyst formation. The time between emergence and cyst formation was from 5 to 20 minutes. Twenty-five cysts measured 0.20 to 0.25 mm in diameter with a mean of 0.23 mm. The encysted stage is infective as soon as fed to suitable definitive hosts. Cysts were also infective if kept in the refrigerator for at least a week.

Infection of Definitive Hosts.—Cysts formed from cercariae emerging from naturally infected snails were fed to 7 ducks, 7 chickens, 2 blue-winged

teals, 5 laboratory rats, 2 meadow mice, and 1 white mouse. A total of 283 worms of various ages were collected from the teals, ducks, and chickens. One immature specimen was found in the laboratory mouse and 4 worms in two of the rats.

The first successful infection resulted from feeding a young blue-winged teal; the second from feeding a merganser duckling; the third from feeding a laboratory-raised duckling. The 3-day-old worms recovered from the cecum of the blue-winged teal showed evidences of 2 remaining eye-spots and a beginning of the branching of the excretory trunks (Fig. 21). The 8-day-old worms taken from the merganser duckling were nearly mature and showed 3 rows of ventral glands. The 19-day-old worms taken from a laboratory-raised duckling appeared fully grown. The heaviest infection was obtained in a young rooster fed about 150 cysts. Seventeen days later 120 mature worms were recovered, of which 84 were found in one cecum and 36 in the other. All ducks and chickens which were fed cysts of this species yielded positive infections in the cecum.

Many mice and rats were fed cysts but only one mouse and 2 rats yielded worms, many immature. The results of feeding experiments with these animals indicate that they are abnormal hosts for this species. Since birds are the most suitable hosts it would be expected that wild waterfowl inhabiting the Douglas Lake region would be expected to be the natural hosts for this species. Eleven specimens of this form were taken from a semi-palmated plover, *Aegialitis semipalmata,* by Dr. Sterling Brackett at Douglas Lake.

The Adult (Figs. 22, 24, 25).—Twenty specimens of whole mounts taken from the cecum of an experimental chicken 13 days after infection were used in making the measurements for the following descriptions:

Notocotylus stagnicolae n. sp.
(Figs. 21–25)

Specific Diagnosis: Adult; characteristics of the genus; body elongate, concave ventrally, muscular, 2.99 ± .18 (2.67–3.40) mm long by 0.83 ± .06 (0.72–0.94) mm wide; anterior half of ventral surface covered with many spines arranged in oblique rows; oral sucker ventral, muscular, 0.16 ± .008 (0.13–0.18) mm; esophagus 0.09 to 0.18 mm in length, narrow, bifurcating anterior to opening of genital pore; intestinal ceca, irregular tubes descending to posterior median border of testes, with dilations and indentations; genital pore ventral, somewhat posterior to intestinal bifurcation; three rows of ventral glands which may be everted and in cleared condition usually show a slit in the surface; numbers in lateral rows from 14 to 17 with a mean of 15, in middle row 13 to 15 with a mean of 14; testes with 8 to 12 lobes along lateral margin, 0.44 ± .04 (0.34–0.56) mm long by 0.24 ± .02 (0.17–0.30) mm wide; distance of testes from posterior border 0.10 ± .06 (0.06–0.14) mm; cirrus echinate, eversible; both internal and external seminal vesicle present; ovary smaller than testes, 0.23 ± .02 (0.20–0.30) mm long by 0.26 ± .02 (0.21–0.30) mm wide, often lobed in outline; vitellaria lateral with about 24 to 34 groups of follicles on each side; position of vitellaria (length of body divided by forward extent of follicles) 1.8 to 2.1 with a mean of 1.9; from the oötype the uterus continues forward in numerous coils most

of which lie intracecal; uterine loops between vitellaria and cirrus sac on side of metraterm 3 to 5 with a mean of 4; seminal receptacle absent but lower portions of uterus containing numerous spermatozoa; excretory vesicle receiving 2 branched excretory tubules joined anteriorly below the oral sucker.

Hosts: Gallus domestica, Anas domestica, Querquedula discors, Mergus merganser (experimental), *Aegialitis semipalmata.*

Habitat: Cecum.

Locality: Douglas Lake, Michigan.

Type specimens: Co-types deposited in the U.S.N.M. Helm. Coll. No. 36839.

Studies in ventral gland relationships were made on 31 adult specimens of *N. stagnicolae* taken from the cecum of a chicken. It was noted that the glands usually showed up as indented oval crevices (Fig. 24). The most common alignment was 14 glands for the median row and 15 for each of the lateral rows. The next most common arrangement was 15 glands for the median row and 16 for each of the lateral rows. The highest total for the 3 rows was 48 of which 14 were found in the median row and 17 in each of the lateral rows. The lowest number observed for the median row was 13 and for the lateral rows 14. The number of glands in the middle rows anterior to the most anterior glands in the lateral rows also varied. Usually one but in many cases 2 glands in the median row were anterior to the most anterior glands in the other two rows.

The adults of *N. stagnicolae* can be differentiated from most other species of the genus *Notocotylus* by the following combination of characters: (1) only 3 to 5 uterine loops extending above the vitellaria; (2) the number of ventral glands in the lateral rows from 14 to 17, in the middle row from 14 to 15; (3) extent of vitellaria slightly anterior to midbody; (4) the number of lobes of testes along lateral border from 8 to 12; (5) measurements of reproductive organs. Because of insufficient descriptions of the adults of some species it is also necessary to use certain larval characters for differentiation.

In reviewing the literature I find 4 species which are so similar to *N. stagnicolae* that differentiation by the use of the adult characters listed above is very difficult. Of these *N. intestinalis* Tubangui, 1932, may be separated from *N. stagnicolae* by its very narrow ovaries and testes; *N. attenuatus* Rud. because the cercariae have a longer body and a shorter tail; *N. magniovatus* Yamaguti, 1934, because the rediae have posterior locomotor appendages; and *N. imbricatus* (Looss, 1896) by having cercariae which are smaller in size measurements.

These comparisons make it evident that no species has been described with characters identical in both larval and adult stages with *N. stagnicolae.* However, because of inconsistent bases of differentiation and incomplete descriptions it is hard to tell whether all the species described in the genus *Notocotylus* are valid and whether *N. stagnicolae* is really different from all of them. Subsequent life history studies may show that several of them are conspecific. Meanwhile, in the absence of better evi-

dence, it is considered preferable to keep the present species names in this genus and add *N. stagnicolae* as a new species.

DISCUSSION

It is interesting to note that the mother sporocyst of one species reported here, *Q. quinqueserialis,* contains four mother rediae and the sporocyst of the other, *N. stagnicolae,* contains only one. In the life cycle of *N. urbanensis,* described by the author in a preliminary note (Herber, 1940), mother sporocysts were found that contained both 6 and 8 mother rediae. Joyeux (1922) found 2 rediae inside the mother sporocyst in the species for which he worked out the life cycle. The rediae seen by Joyeux inside the mother sporocysts were probably mother rediae although he thought that there was only one generation of rediae in this life cycle. Several other workers, Looss (1896), Wesenburg-Lund (1934), and Yamaguti (1938), reported that they found 2 generations of rediae. The life cycle which Joyeux described is strikingly different from the others in this group since the cercariae have rudimentary tails and encyst without escaping from the snail intermediate host. All other cercariae described from this group have long tails, are active swimmers, and encyst on vegetation or on the snail shell after emerging from the intermediate host.

The theory that cysts of notocotylids need a certain time for maturing before they become infective was brought out by Harper (1929). The experiments reported here seem to indicate that metacercariae are infective immediately after encystment. Whether Harper's species is unusual in requiring such an interval cannot be determined without more experimental work.

The identification of adults of the subfamily NOTOCOTYLINAE and especially in the genus *Notocotylus* presents a great many difficulties because there is considerable similarity of structure and few good characters for differentiation. The following characters have been emphasized by various workers in separating them: size and shape of body, number and arrangement of ventral glands; size of organs, position and detailed structure of organs. Some of these characters are evaluated here in their relation to species differentiation.

Body size has very limited use as it is dependent upon several factors such as maturity, host relationship, state of contraction at the time of examination, and method of fixation. This character can only be used in separating species where variations are quite pronounced.

Ventral gland relationships are of some value as diagnostic characters. Since methods of fixing and mounting specimens may completely obscure them, the statement that they are absent in the description of a species should be viewed critically unless it is certain that proper methods of

study were used. Also, for the same reason the number and arrangement of these glands are often given incorrectly in species descriptions. The presence of 5 rows of these glands instead of 3 is of course a good character which gave the basis for the separation of the genus *Quinqueserialis* from *Notocotylus*. In my studies a considerable variation in number and arrangement was found which would have to be taken into consideration in comparing closely related species. It would seem, therefore, that the ventral gland relationship has a somewhat limited value in distinguishing closely related species of the genus *Notocotylus*.

The size of eggs has been used as a means of distinguishing species regardless of age or method of fixation. In my opinion egg-size is of little value as an aid in differentiating species but differences in length of egg filaments fixed soon after ovipositing are probably significant.

The outline of the testes of most species of *Notocotylus* is smooth along the median border but the lobing along the lateral borders can be used as a diagnostic character. Testes and ovary measurements can be used as aids in diagnosis only in a limited number of species and in series of worms of approximately the same age.

The number of uterine loops anterior to the vitellaria is dependent upon the range of the vitelline follicles. In most species the posterior limits of the vitellaria are immediately in front of the anterior limits of the testes but the forward extent varies because of the difference in the number of follicles. Therefore, the number of uterine loops anterior to the vitellaria and the position of the vitellaria are good diagnostic characters.

Larval characters have not been used extensively and have a limited value in distinguishing species of NOTOCOTYLINAE because of the small amount of work on life cycles. Body and tail size differences are often useful in distinguishing different species of cercariae. It has also been found that the relation of the anterior transverse portion of the excretory tubules to the median eye-spot is a very good diagnostic character. If the cercariae are allowed to encyst normally the diameter of such cysts is constant enough to use it as a means of distinguishing species. Size differences in body, tail, and cyst were utilized by L. and U. Szidat (1933) in distinguishing cercariae which when fed to suitable hosts produced different species of adults. Rothschild (1938) confirmed the value of such size differences in cercariae and also noted differences in the structure of the anterior transverse portion of the excretory tubules. The amount of pigmentation and time of encystment aids only as a sort of preliminary identification of cercariae. Rediae in this group usually have no posterior locomotor appendages and are much alike in the different species. In four species for which mother sporocysts are known they contain different numbers of rediae.

Certain of the earlier authors considered as separate species adults

found in different hosts. This is obviously unsound as one species may develop in a wide variety of animals. For example, *N. stagnicolae,* a bird form, has developed to maturity in mammals. It was suggested by L. and U. Szidat (1933) that specificity is very marked in the case of the snail host. This view is confirmed in my life history studies. Although the degree of this specificity is not known at present, it is possible that it may have real significance.

SUMMARY

The life histories of *Quinqueserialis quinqueserialis* (Barker and Laughlin, 1911) and *Notocotylus stagnicolae* n. sp. have been completed experimentally.

The definitive hosts of *Q. quinqueserialis* are *Ondatra zibethicus, Microtus pennsylvanicus,* and *Zapus hudsonius.* When eggs of this parasite were placed with the intermediate host, *G. parvus,* infection occurred and mother sporocysts could be recognized 9 days afterwards. Mother sporocysts of this species contain 4 mother rediae. About 20 days after exposure to infection daughter rediae were found in the digestive gland of the snails. About 23 days after infection immature cercariae emerged from the daughter rediae and migrated into the digestive gland tissues where a short period of maturation occurs. Mature cercariae were found 26 days after infection. They emerge from the snail and usually encyst on vegetation. Meadow mice become infected when fed metacercariae of this form. These flukes develop to maturity in about 2 weeks. Descriptions are given of all stages in the life cycle.

The definitive hosts of *N. stagnicolae* n. sp. are *Gallus domestica, Anas domestica, Querquedula discors, Mergus merganser,* and *Aegialitis semipalmata.* Eggs of this parasite are infective when placed with the snail host, *S. e. angulata.* The course of the development in the intermediate host is very similar to that of *Q. quinqueserialis* except that the mother sporocyst of this form contains only one mother redia. In the definitive host these flukes mature in about 10 days. All stages of the life cycle of this trematode are also described.

The following characters were found to be most helpful in distinguishing different species of the NOTOCOTYLINAE: (1) the number of ventral glands in each row, (2) the anterior extent of the vitellaria, (3) the lateral lobation of the testes, (4) the body and tail size of cercariae, and (5) the course of the excretory tubules of cercariae. A discussion is given of the value of the different characters which have been used to differentiate the species of NOTOCOTYLINAE.

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Plate I

Stages in the life cycle of *Quinqueserialis quinqueserialis*
(All drawings made by use of camera lucida)

Fig. 1. Mother sporocyst in mantle of snail, 16 days after exposure to infection.
Fig. 2. Mother sporocyst found free after crushing snail, 18 days after exposure to infection.
Fig. 3. Mother redia.
Fig. 4. Daughter redia.
Fig. 5. Cercarial embryo.
Fig. 6. Elongated cercarial embryo.
Fig. 7. Developing cercaria.
Fig. 8. Body and part of tail of mature cercaria.
Fig. 9. Metacercaria, encysted stage.
Fig. 10. Adult, ventral view.
Fig. 11. Egg.
Fig. 12. Outline of adult showing arrangement of ventral glands.

Plate II

Stages in the life cycle of *Notocotylus stagnicolae* n. sp.
(All drawings made by use of camera lucida)

Fig. 13. Mother sporocysts showing attachment along edge of snail intestine, 14 days after exposure.
Fig. 14. Mother sporocyst showing young mother redia, 16 days after exposure.
Fig. 15. Mother sporocyst showing almost mature mother redia, 16 days after exposure to infection.
Fig. 16. Mother sporocyst showing mature mother redia ready to break through the wall of mother sporocyst, 18 days after exposure to infection.
Fig. 17. Mother redia, 57 days after exposure.
Fig. 18. Redia containing cercariae, natural infection.
Fig. 19. Mature cercaria.
Fig. 20. Encysted metacercaria.
Fig. 21. Immature adult, 3 days after feeding.
Fig. 22. Adult, ventral view.
Fig. 23. Egg.
Fig. 24. Outline of adult showing arrangement of ventral glands.
Fig. 25. Outline of posterior ends of three adults, showing lobation of testes.

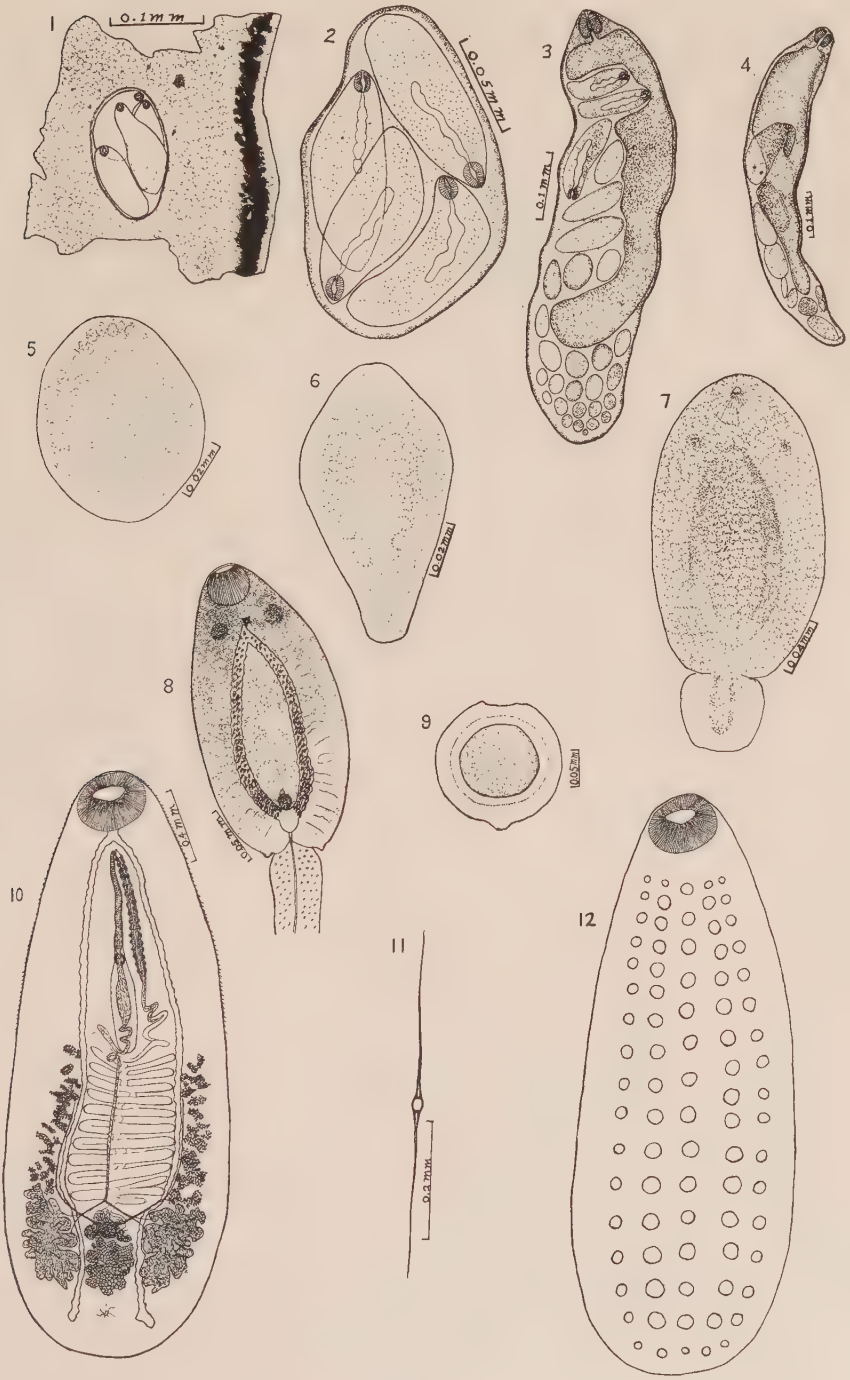
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PLATE I

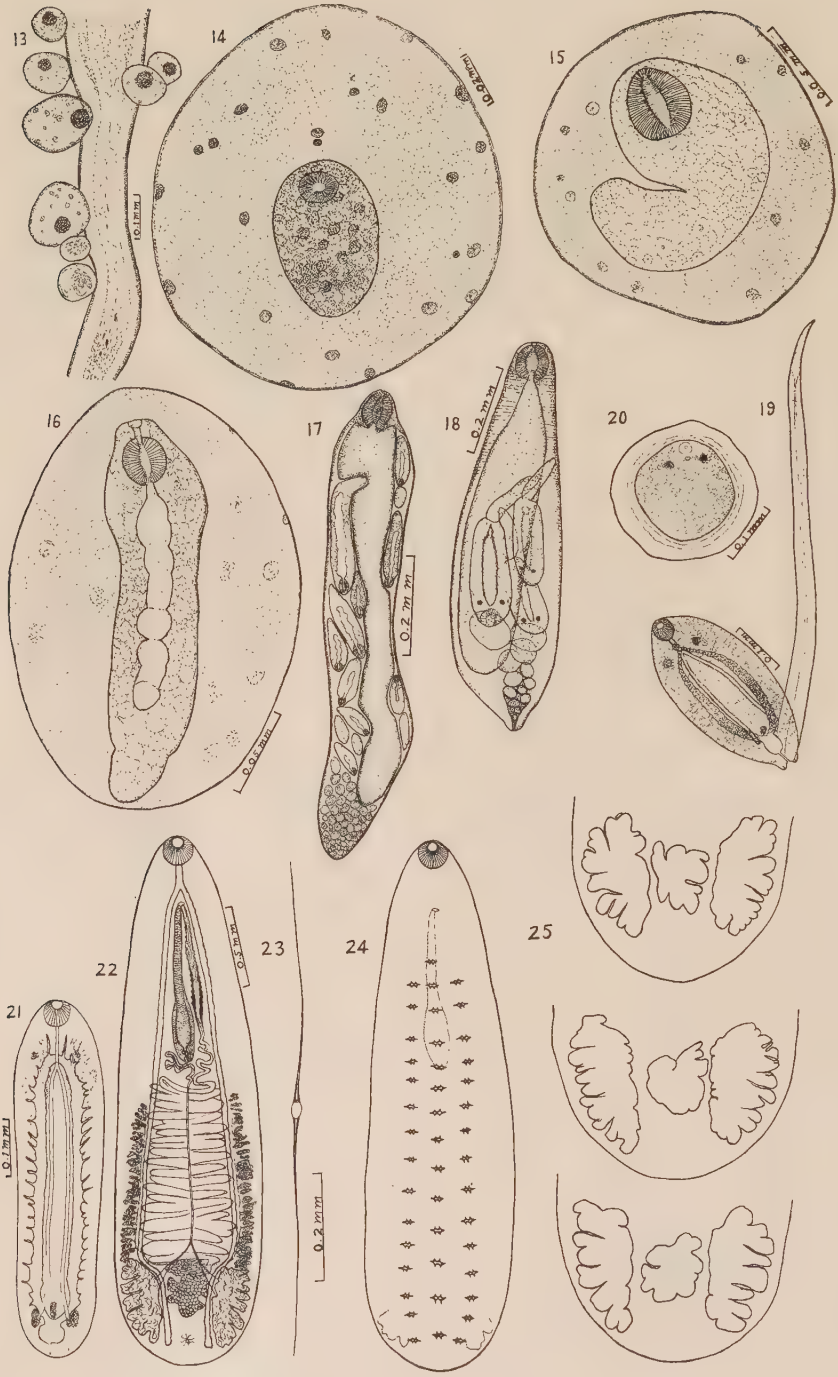

PLATE II

ACTIVE IMMUNITY IN MICE AGAINST *TRICHINELLA SPIRALIS*

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The acquisition of immunity against *Trichinella spiralis* through recovery from a prior infection with the homologous parasite has been demonstrated in the rat (1), the guinea pig (2), the pig (3), and the monkey (4). Most investigators are agreed that the acquired immunity is directed largely against the intestinal phase of the parasite, although the precise mechanism of the immunity is still obscure. Some believe the immune response is made chiefly or entirely by the tissues of the intestinal wall itself, and that the tissues of the remainder of the body have comparatively little or nothing to do with the immune process. An increased secretion of mucus and increased intestinal peristalsis are presented by those supporting this view as the chief factors operative in the immunity. These workers generally have failed to demonstrate either the passive transfer of immunity with the serum or the development of an inflammatory reaction in the intestinal wall of the immune host, and much of their contention is based upon these negative observations (5). Other investigators, however, consider that a generalized immune response must be made to the parasite before an animal can acquire a substantial degree of immunity, although they feel that this response may, and probably does, act upon the parasite only in strategically disposed sites, of which the intestine is one.

In the present paper, the results are offered from a study of acquired immunity to trichiniasis in the albino mouse. Strangely, the mouse has been but little employed in experimental trichiniasis. One of the few reports in which this animal has been used is that by the author and Kaplan of some years ago in which it was shown that rabbit antiserum rich in the specific antibody conferred to mice a partial immunity against trichiniasis (6). It is now shown that mice become actively immune to *Trichinella spiralis* as a result either of prior infection with the parasite or of vaccination with its antigens. The results are offered with a view to throwing some further light upon the mechanism of immunity in this disease.

GENERAL METHODS

The Method of Infecting Mice.—The larval worms were obtained from the muscles of infected rats, by the method to be described presently. After the larvae were freed of all rat tissue, they were suspended in bac-

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teriological nutrient broth to which 20 per cent of gelatin was added. In this medium, the worms not only survived for several days, but also remained in relatively stable suspension for some minutes, thus facilitating the administration of approximately identical doses of larvae to each mouse of a series being infected. Known numbers of worms, usually in a fluid volume of 0.1 cc, were delivered by syringe through a blunted 22-gauge hypodermic needle lowered carefully into the retropharynx of each mouse. Usually the mice swallowed the fluid, and with it the worms, with no trouble.

The Recovery of Adult Worms from the Intestine.—From four to seven days after larvae were fed to certain of the mice, the animals were killed with ether and autopsied. The small intestine was placed in physiological salt solution and slit lengthwise with small scissors. The intestine was then agitated so as to free the living, moving worms into the suspending fluid. The whole of this fluid was examined at once for the presence of adult worms, small portions being transferred to Syracuse watch glasses and searched with a binocular dissecting scope ($\times 7$, $\times 20$). The intestine was agitated in fresh amounts of salt solution as required, search being continued until no further worms were recovered.

The Recovery of Larval Worms from Muscle Tissue.—From 27 to 35 days after infection, the animals were killed with ether, skinned, and eviscerated. The remaining carcass was then ground in an ordinary meat grinder. The ground tissue was weighed and suspended in 0.5 per cent hydrochloric acid (at 37° C) to which 1.0 per cent powdered pepsin was added. Thirty cubic centimeters of this fluid were used per gram of mouse tissue. The mixture was placed in an incubator at 37° C and agitated constantly until all the meat was digested (usually within four hours). When digestion was complete, samples of the fluid were transferred to Syracuse watch glasses and the number of larval worms determined by direct count with the aid of a low-power binocular microscope ($\times 7$, $\times 20$). Finally, the number of worms in the total fluid volume, and thus in the entire carcass, was established by calculation.

THE ACQUISITION OF IMMUNITY THROUGH PRIOR INFECTION

Mice were fed one or more preliminary doses of living *Trichinella* larvae, and then, after an interval, were fed, together with control mice, a test dose of larvae. Some of the animals were autopsied after from five to seven days, and the adult worms in the intestine were recovered. Other mice were let live for from 27 to 35 days. The number of larval worms recoverable from the muscle tissue of these animals was then determined.

The Development of Adult Worms in the Intestine.—Two groups of mice were used to determine whether or not prior infection with *Trichi-*

nella prevented larval worms fed subsequently from developing to adults. Group A (5 mice) had received 100 larvae 22 days before the test feeding of 175 larvae. Group B (6 mice) had received 100 larvae 60 days before and 500 larvae 30 days prior to the test feeding of 250 larvae. The animals of Group A, with 7 control mice, were autopsied 5 days, and those of Group B with 5 controls 7 days, after the test feeding.

In the case of none of the previously infected mice were so many adult worms recovered as were found in the control mice. From the 5 previously infected animals of Group A, 1, 3, 4, 23, and 45 worms were recovered, representing an average of 8.6 per cent of the larvae fed. From Group B, 0, 0, 1, 1, 2, and 17 adults were recovered, representing 1.4 per cent of the worms fed. From the control mice for Group A, in contrast, 65, 82, 83, 101, 102, 108, and 144 (averaging 55.8 per cent) and, from those of Group B, 61, 96, 137, 146, and 197 (averaging 50.8 per cent) of the larvae fed were recovered as adult worms.

The Presence of Larval Worms in the Muscles.—In order to test for the effect of previous infection upon the amount of muscle invasion by larval *Trichinella,* a group of 17 mice were fed four doses each of 250 larvae, given, respectively, 60, 36, 22, and 10 days before a test dose of 1000 larvae. Nine control mice were also fed the test dose. All the mice which survived the test dose, including controls, were killed from 27 to 35 days after infection, and the number of larval worms in the muscles determined.

The muscles of the control mice harbored individually 110,250, 117,-605, 119,475, 129,724, 152,530, 163,213, 174,440, 180,964, and 243,436 larvae, or on the average, 154,626 larvae. The muscles of the previously infected group in contrast, yielded 12,768, 16,966, 25,979, 39,712, 62,216, 101,500, 161,468 larvae, or, on the average, 60,087 larvae. Thus, the controls suffered approximately two and one-half times as much muscle invasion as the previously infected animals, in spite of the fact that the total number of infective larvae fed the controls was but one-half that fed the "immunized" mice. Indeed, the number of larvae recovered from the immunized group is only little more than the number expected as a result of the initial immunizing dose of 250 larvae. (Compare average of 60,087 for these immune mice with 55,139 for control mice for the vaccinated animals, which were given 250 larvae.) Thus, following the initial dose, these mice must have been almost perfectly protected against muscle invasion.

By the third day subsequent to the test dose, many of the previously infected mice revealed an exudate coming from the anus. When this exudate persisted for several days, death occurred. Altogether, 10 of the 17 previously infected mice died, the deaths occurring from the 9th to 23rd day after the test infection. Not any of the control mice pre-

sented this symptom, and none died during this period. On the other hand, the surviving mice of the previously infected group, continued to add weight, following an initial decline immediately after the test feeding, and appeared to suffer not at all from the infection. The control mice, in contrast, lost little or no weight until during the fourth week after the test dose. When autopsied, these animals were rapidly losing weight and some were moribund. The ultimate survival of any of the control mice was unlikely.

THE ACQUISITION OF IMMUNITY THROUGH VACCINATION

Earlier workers have endeavored to protect animals against *Trichinella spiralis* by vaccination with the antigens of the homologous parasite. Rats can generally be protected by such vaccination (7), although efforts thus to protect hogs have failed (3). The effect of parenteral vaccination upon the resistance of mice was tested in an effort to determine whether immunity could be developed in this animal in the absence

TABLE 1.—*Summary of data. Average number of adult or larval* Trichinella spiralis *recovered from immunized and from control mice*

No. of mice used	Method of immunization	No. of larvae fed as test dose	Days till autopsy after test dose	Average No. of adults recovered from intestine	Average No. of larvae recovered from muscles
5	Prior infection (a)	175	5	15.2	–
6	" " (b)	250	7	3.5	–
7	Control	175	5	95.0	–
5	"	250	7	127.4	–
7	Prior infection (c)	1000	27–35	0.0	60,087
9	Control	"	"	0.0	154,626
11	Vaccination (d)	250	4	71.5	–
10	Control	"	"	132.0	–
9	Vaccination (d)	250	29	0.0	20,248
7	Control	"	"	0.0	55,139

(a) 100 larvae feed 22 days before test infection.
(b) 100 larvae fed 60 days, and 500 larvae fed 30 days before test infection.
(c) 250 larvae fed 60, 36, 22, and 10 days before test infection.
(d) Eight injections, given on alternate days, of one-tenth cc of 1 per cent suspension of *Trichinella spiralis* powder in carbolized salt solution per 10 gm body weight.

of previous intestinal infection with this parasite. The effect both on the development of adult worms in the intestine of previously vaccinated mice and upon the larval invasion of their musculature was determined.

The mice were vaccinated according to the following procedure: one-tenth cc of 1 per cent suspension of *Trichinella spiralis* powder in carbolized salt solution was administered per 10 gm mouse body weight. Eight injections were given on alternate days.

The Development of Adult Worms in the Intestine.—Five days after the last injection of vaccine, eleven mice, along with ten control animals, were fed 250 larvae. Four days later, all the animals were autopsied, and their small intestines searched for adult *Trichinella*.

The vaccinated mice yielded only about half as many worms as did the normal mice. From the vaccinated mice, 29, 30, 34, 35, 43, 50, 85, 88, 90, 125, and 178 adults were recovered. From the controls, 55, 103, 105, 113, 116, 120, 123, 166, 188, and 231 adults were recovered. Expressed as percentages of the larvae fed, the average yields by the vaccinated and control groups were, respectively, 28.4 per cent and 52.8 per cent. Two of the eleven vaccinated animals had, evidently, failed to respond to vaccination, but all of the nine remaining mice acquired a significant degree of immunity.

The Presence of Larval Worms in the Muscles.—To determine the relative amount of muscle invasion with larval *Trichinella* in vaccinated and control mice, 9 vaccinated and 7 normal animals were fed 250 larvae on the fifth day after the last administration of vaccine. After from 28 to 29 days, all the mice were autopsied and their muscles digested, as described earlier.

The nine vaccinated mice yielded 4,290, 6,396, 11,040, 11,647, 14,840, 17,189, 26,880, 44,800, and 45,156 larvae—an average of 20,248 larval worms. The control animals, in contrast, yielded 23,545, 49,800, 56,832, 57,426, 57,456, 59,640, and 81,280 larvae—an average of 55,139 larvae. Most of the vaccinated mice were rather highly immunized, apparently, as a result of vaccination, although two animals yielded almost as many larval worms as the control mice.

DISCUSSION

From the data offered in this paper, it is clear that immunity is engendered by mice against *Trichinella spiralis* both after prior infection with this parasite and after vaccination with its antigens. It is particularly noteworthy that, after immunization by either method, the immunity is effective against the intestinal phase of the parasite. The same finding has been reported previously after passively immunizing mice against this parasite (6). As may be expected, however, a higher level of immunity is developed by infection with *Trichinella* than by vaccination or by passive immunization. However, this quantitative difference by no means implies that the immune response after infection is qualitatively distinct from that after the two less effective procedures.

One matter of considerable interest in the results offered is the death, a few days after the test dose, of some of the mice immunized by prior infection. These animals suffered acutely from the test dose beginning about three days after its administration. The control animals at this time were not noticeably affected. Evidently the immune animals manifested a hypersensitive response to the *Trichinella* substance. The presence of an exudate in the intestine of the immune mice during these days supports this view, suggesting that an inflammatory response had been

initiated. Unfortunately, however, the response to the infection became so violent that many of the mice succumbed. Similarly, acute early symptoms, suggestive of hypersensitivity to the *Trichinella* substance, have also been observed in cases of human trichiniasis which have suffered reinfection (8). The capacity of highly immune animals to make such dangerous or even fatal hypersensitive responses is, of course, well known in tuberculosis and many other infectious diseases.

SUMMARY

Mice become partially immune to *Trichinella spiralis* as a result of prior infections with the specific parasite. They likewise manifest an immune response to this form after repeated vaccination with the antigens of the homologous worm. The immunity against *Trichinella* is directed particularly against the intestinal phase of the parasite, no matter whether the immune response is engendered by prior infection or by vaccination.

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PASSIVE TRANSFER OF IMMUNITY TO *TRICHINELLA SPIRALIS* IN THE RAT

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Rats have been shown to acquire immunity against *Trichinella spiralis* after a preliminary infection with this parasite (1) or after vaccination with its antigens (2). The attempts thus far to demonstrate passive immunity in the rat, however, have failed, although antibody has been detected in the serum of immune rats (3), and larval worms immersed in such serum generally lost their infectivity (4). It has also been shown that immune mother rats transfer immunity to their young (5), although the precise route of transfer and the significance of the serum antibody in such transfer is not as yet established.

The present paper offers results from a study designed to test for passive transfer of immunity to *Trichinella spiralis* in the rat, using the serum of immune rats. The tests have been performed in the 10-day-old nurslings of normal mother rats.

GENERAL METHODS

Methods of infection of animals, recovery of adult *Trichinella* from the intestine, and recovery of larvae from muscle were the same as used in previous work with mice (6). For the preparation of immune serum, four adult normal rats were fed 2000 isolated larvae of *Trichinella spiralis*. After one month, these animals were bled and their serums pooled for use in the present work. Several normal animals were bled to provide the normal serum with which some of the control rats were injected. Five-tenths cubic centimeter of these serums were administered intraperitoneally on the two days prior to and on the day following infection. The dose given represented 3 cc per 100 gm weight of the recipient.

RECOVERY OF ADULT *Trichinella* FROM THE INTESTINES OF PASSIVELY IMMUNIZED RATS AND FROM CONTROL RATS

The first experiment was designed to determine whether the administration of an immune serum to an animal affected the development of the larval worms fed to this animal. Twenty-one 10-day-old rats nursing normal mothers were used in this study. Eight were injected with immune serum, and 7 with normal serum according to the schedule previously described. These rats, together with the six remaining animals which were given no serum, were then each infected with 250 larvae.

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Four days after infection, all the animals were killed with ether and their small and large intestines searched for adult *Trichinella*.

The rats which were injected with immune serum yielded distinctly fewer adult *Trichinella* than did those given normal rat serum, or those kept as untreated controls. Those given immune serum yielded 3, 5, 7, 8, 9, 16, 16, and 26 adult worms. Those given normal serum yielded 17, 40, 65, 66, 71, 79, and 83 adults. Those given no serum yielded 38, 44, 52, 73, 78, and 86 adult worms. On the average, only 4.5 per cent of the larvae fed were recovered as adults from the passively immunized group, whereas 24.0 per cent and 24.7 per cent were recovered, respectively, from the group given normal serum and from the untreated group.

RECOVERY OF LARVAL *Trichinella* FROM THE MUSCLES OF PASSIVELY IMMUNIZED RATS AND FROM CONTROL RATS

The second experiment was designed to determine the number of larval *Trichinella spiralis* in the muscles of immunized rats compared with control animals. Fourteen 10-day-old rats nursing two normal mothers were used for this work. Seven of these were given immune rat serum, and four were given normal serum intraperitoneally according to the previously described schedule. The three remaining rats were given no serum. Each of the animals was fed 250 isolated larvae. Four weeks after infection the rats were killed with ether, skinned, and eviscerated. The remaining carcass was digested and the total larval yield determined.

Fewer larvae were obtained from the muscles of passively immunized rats than from the control animals. From those given immune serum, 3,125, 6,234, 6,318, 13,248, 14,458, 18,414, and 22,830 larvae were recovered. From those given normal rat serum 36,630, 46,189, 49,369, and 58,266 larvae were obtained. From those given no serum 26,964, 51,129, and 91,600 larvae were found. On the average, 12,089 larvae were recovered from the muscles of the animals given immune rat serum, whereas from those given normal serum and from the untreated controls the average yields were, respectively, 47,613, and 56,564.

TABLE 1.—*Summary of data. Average recovery of adult* Trichinella *from intestine and larvae from muscles of passively immunized mice and from controls*

No. of rats in group	Rat serum given	Days till autopsy after test dose	Average No. of adults recovered from intestine	Average No. of larvae recovered from muscle
8	Immune	4	11.2	–
7	Normal	4	60.0	–
6	None	4	61.7	–
7	Immune	28	0	12,089
4	Normal	28	0	47,613
3	None	28	0	56,564

Test dose of larvae: 250 isolated larvae.
Administration of serum: 0.5 cc intraperitoneally for two days before, and one day following infection.
All rats 10 days old at start of experiment.

DISCUSSION

The results presented indicate that the immunity actively acquired by rats against *Trichinella spiralis* can be passively transferred to normal rats by transfer of the serum. The passively immunized animal evidently checks the infection in the same manner as does the actively immunized rat—namely, by limiting the development of adult worms in the intestine. This developing stage of the parasite is evidently the one most vulnerable to the immune effect. The limitation upon development is less nearly perfect in passively immunized animals than in animals immunized by previous infection, as would be expected. The comparatively small amount of serum transferred (3 cc per 100 gm by body weight on three days)—especially following the dilution which this experiences in the body of the recipient—could hardly equal in its effect upon *Trichinella* that of the total blood of an animal actively immunized by prior infection. Nevertheless, there is no reason to suspect that this difference in effect has a qualitative basis.

In the present experiments, the demonstrated immunity is undoubtedly conferred by some substance in the transferred serum. This humoral substance—which is perhaps antibody—would certainly be as effective in the animal which developed it as it is after transfer to a normal animal. Therefore, the same humoral immune mechanism operative after passive transfer must act likewise in actively immunized animals and be at least in part responsible for their acquired immunity. In both actively and passively immunized animals, there is ample opportunity for the humoral substance to come in contact with the developing parasite, since the larval *Trichinella* characteristically bores between the villi and into the intestinal glands to feed on the intestinal mucosa (7).

It is felt by some that the immune response in trichiniasis is made so promptly that the usual (humoral antibody) defense mechanism could hardly be responsible. It is pointed out that actively immunized rats expel *Trichinella* larvae in from 3 to 18 hours after these are ingested (8). Yet, it should be remembered that an Arthus reaction becomes evident in the skin of a well sensitized rabbit within two or three hours after a specific antigen is injected, even if the animal be passively sensitized (9). Furthermore, fatal anaphylactic shock may occur within a few minutes after the homologous antigen is injected into a well-sensitized guinea pig, again, no matter whether the animal be actively or passively sensitized. Finally, allergic reactions in the skin as well as other tissues (e.g., intestine) of man are typically immediate responses, reaching their peak within a few minutes after the tissue has had contact with the specific antigen. Thus the rapidity of the immune response in trichiniasis is not without precedent in immunology, and the conventional mechanisms, cannot, therefore, be excluded as the basis of immunity in this infection.

CONCLUSIONS

Immunity to *Trichinella spiralis* can be passively conferred upon normal rats by transferring to them serum from a rat immunized by prior infection. As might be expected, the immune response of passively immunized rats is less powerful than that generally manifested by actively immunized animals.

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AGE RESISTANCE OF THE ALBINO RAT TO *CYSTICERCUS FASCIOLARIS*

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Although age resistance has been demonstrated in many protozoan infections as well as in infections with a number of nematodes, this phenomenon has been observed with only very few cestode parasites. It has been described for *Hymenolepis nana* in man (1), as well as for its rodent variant, *Hymenolepis fraterna,* in rats and mice (2). The chicken is known to manifest age resistance to *Raillietina cesticillus* (3), as is also the herring gull to a species of *Diphyllobothrium* (4). The development of natural resistance with age has likewise been suggested for the rat against *Cysticercus fasciolaris* (5), the larval stage of the cat tapeworm *Taenia taeniaeformis.*

The possibility of the manifestation of age resistance by rats to *Cysticercus fasciolaris* has been reinvestigated by the author, and the results of this study are offered in the present paper. It has been found that older rats rarely develop significant infections with this parasite, whereas younger animals—if past the weaning age—suffer heavy infections. However, nursling young, even if born of normal mother rats, are like old rats comparatively or wholly refractory to this parasite.

GENERAL METHODS

The infection and examination of rats.—Rats (Sherman strain) of known age were infected by mouth with 500 onchospheres of *Taenia taeniaeformis* obtained by opening a gravid segment of the parasite collected freshly from a laboratory cat. The onchospheres, suspended in 0.1 cc of physiological salt solution, were delivered by syringe through a blunted 22-gauge hypodermic needle lowered carefully into the retropharynx of each animal. The rats were autopsied 28 days after infection, and the number of living and of dead cysticerci counted on the surface of their livers.

PROCEDURE AND RESULTS

Altogether 144 rats of age groups ranging from newly born to 11 months old animals were used in this experiment. Approximately equal numbers of animals of each sex were included in the different groups.

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The results of the cyst counts made at the autopsy of 54 representative animals, all of which were infected simultaneously from the same suspension of onchospheres, are presented in Table 1, and photographs of the

TABLE 1.—*Number of cysticerci counted in rats when autopsied 4 weeks after infection per os with 500 onchospheres of* Taenia taeniaeformis

Rat number	Sex	Age when infected	Number of cysts recovered		
			Living	Dead	Total
1–4	♀	New-born	0	0	0
5–8	♂		0	0	0
9–11	♀	7 days	0	0	0
12	♀		0	1	1
13	♀		0	3	3
14–16	♂		0	0	0
17	♂		0	1	1
			0	*0.6*	*0.6 Av.*
18	♀	15 days	3	64	67
19	♀		0	41	41
20	♀		0	73	73
21	♂		1	52	53
22	♂		38	70	108
23	♂		26	117	143
			11.2	*69.5*	*80.8 Av.*
24	♀	25 days	103	0	103
25	♀		185	0	185
26	♀		208	0	208
27	♂		126	0	126
28	♂		110	0	110
29	♂		195	0	195
			154.5	*0*	*154.5 Av.*
30	♀	45 days	13	130	143
31	♀		64	36	100
32	♂		77	5	82
33	♂		73	0	73
			56.7	*42.7*	*99.5 Av.*
34	♀	60 days	80	82	162
35	♀		34	126	160
36	♀		5	83	88
37	♂		16	64	80
38	♂		7	59	66
39	♂		44	52	96
			31	*77.6*	*108.6 Av.*
40	♂	4 mos.	0	141	141
41	♂		13	77	90
42	♂		27	123	150
43	♂		0	11	11
			10	*38*	*98 Av.*
44	♀	6 mos.	0	28	28
45	♀		0	58	58
46	♀		0	17	17
47	♀		0	27	27
			0	*32.5*	*32.5 Av.*
48	♀	11 mos.	0	50	50
49	♀		0	59	59
50	♀		0	151	151
51	♂		0	29	29
52	♂		0	39	39
53	♂		0	17	17
54	♂		0	28	28
			0	*53.2*	*53.2 Av.*

isolated liver lobes of a single representative of the different age groups are shown in Fig. 1.

The rats 25 days old when infected were most susceptible to this parasite. Many cysts developed in these animals and essentially all were

living when the host was autopsied. In rats of older groups, progressively fewer cysts developed, and, of these, only part were alive when the host was autopsied. No living cysts whatsoever were recovered from rats six months old or more when infected.

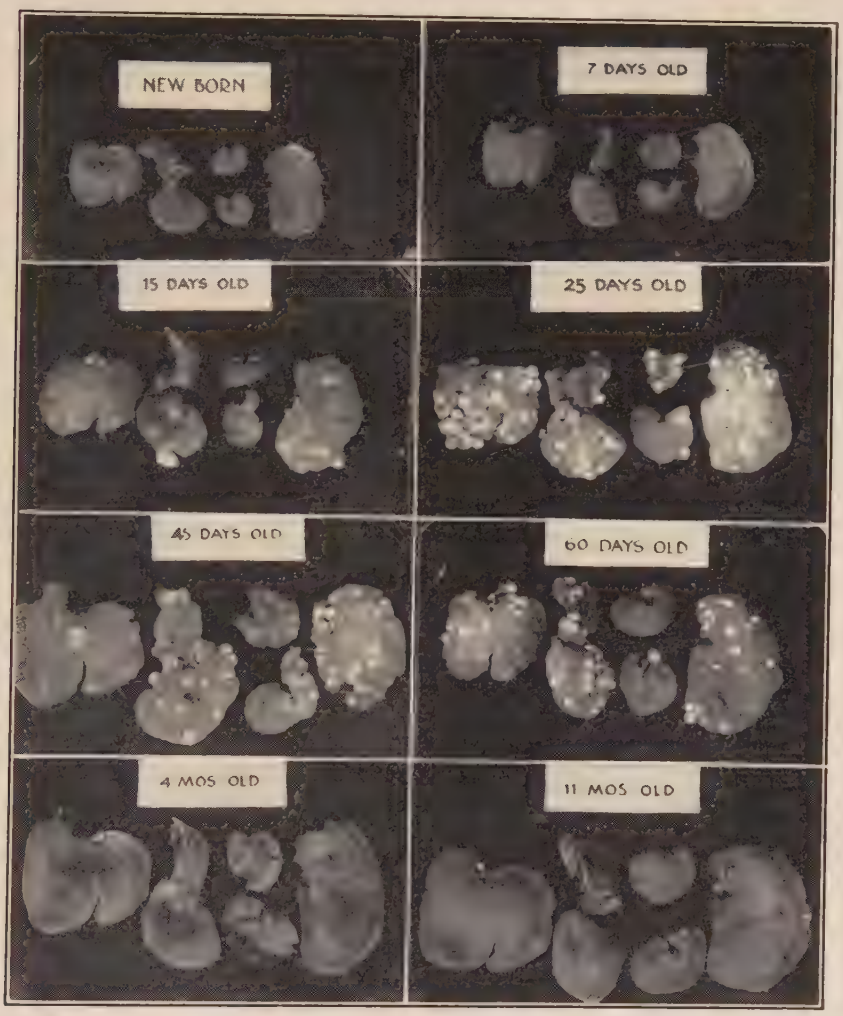

FIG. 1. Photographs of liver lobes of representative rats of the designated age groups autopsied four weeks after being fed 500 onchospheres of *Taenia taeniaeformis*, showing the number of *Cysticercus fasciolaris*.

Among the rats of groups less than 25 days old when infected, progressively fewer cysts were observed as the age group of the host decreased. Rats infected on the day of birth revealed at autopsy 4 weeks later no cysts whatsoever. A few dead cysts but no living ones were found when rats seven days old at infection were autopsied. A number

of living cysts and many dead ones were found in the rats infected at 15 days of age. The mothers of these animals were also autopsied and found to be uninfected.

DISCUSSION

The results given indicate that rats 25 days old are most susceptible to infection with *Cysticercus fasciolaris*. This high susceptibility is preserved largely until the animals reach two months of age. Thereafter, however, the natural resistance of rats increases markedly and those four months old or more usually prevent wholly the maturation of cysticerci, even though the parasite establishes itself initially in the livers of these animals. Curtis, Dunning, and Bullock (5) observed that the percentage of animals which was successfully infected varied significantly with the age at which the onchospheres were administered. From experiments carried out on large numbers of animals, using standard suspensions of *Taenia* eggs, they found that 76% of rats one month old were susceptible to infection, while only 19% of those four months of age showed cysts. However, under certain circumstances, mature cysts may develop in older animals. Miller (6) has reported, in a small group of albino rats 170–172 days of age when infected, an average of 22 living and 19 dead cysts. A heavy suspension of 900 onchospheres was fed in this case because a large number of the eggs were immature. Although mature cysts were present in these animals over five months of age, there is no means of evaluating the significance of such results in view of the fact that data upon animals of other age groups infected with a similar suspension is not available.

The remarkable resistance of the nursling rats is, as yet, not explained. The possibility certainly exists that an inhibitory substance possessed by their normal mothers has been transferred to the young either through the placenta or through the milk. Since precautions were taken to exclude the possibility of accidental infections of the mothers, we believe that these substances are not the specific immune bodies which Miller (7–8) has shown to be transmitted from immune mothers to young. On the other hand, the intestine of the very young rats may lack the enzymes required to free the embryos by digesting the hard and thick envelope of the administered onchospheres. The fact that some development goes on in the older nurslings, even though the cysts generally die or attain only small size, seems to favor the view that the mother rat does transfer an inhibiting substance of perhaps a non-specific character to the young. Such transfer is by no means proved, however, by the data thus far obtained.

SUMMARY

An age resistance is described for the rat against *Cysticercus fasciolaris*. Rats from 25 to 60 days old are more susceptible to this parasite

than are younger or older rats. Newly born rats can seldom be infected at all, and older nurslings yield chiefly dead cysts. Rats infected when four months old or more likewise seldom yield mature cysts, although dead cysts are usually observed in these animals.

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STUDIES ON SOME GORDIACEA OF NORTH AND SOUTH AMERICA*

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Few studies on American Gordiacea have been made since the works of Montgomery (1898–1907) and May (1919). The present paper deals with some North and South American species studied in the light of recent revisions of the group especially by Heinze (1934–1937).

HISTORICAL

Linnaeus (1766) erected the genus *Gordius* for certain species now included in the class Gordiacea and the nematode families Filariidae and Mermithidae. Gmelin (1788) divided the genus *Gordius* into *Gordius* and *Filaria.* Dujardin (1842) named the genus *Mermis* and considered it belonged among the nematodes. Creplin (1847) separated the genus *Chordodes* from *Gordius.* Villot in his monograph (1874) referred all 34 of the then known species of Gordiacea to *Gordius,* but *Chordodes* was accepted and clearly defined by Janda (1893). Camerano (1897) added the genera *Paragordius* and *Parachordodes.* Camerano's (1915) revision listed 113 species which he considered valid. Most of the taxonomic studies of Gordiacea in America have been made by Montgomery (1898–1907). May (1919) described the families Gordiidae and Chordodidae and studied the life cycles of *Gordius robustus* Leidy and *Paragordius varius* (Leidy). G. W. Müller (1927) split the genus *Parachordodes* into *Parachordodes* and *Gordionus.* Heinze (1933–1937) erected the genera *Beatogordius* (1934), *Paragordionus* (1935), *Chordodiolus* (1935) and *Euchordodes* (1937), and four subfamilies (1935) of Chordodidae. Heinze (1937) also developed new techniques in preparing specimens (especially the cuticula) for study. Dorier (1930) showed that larvae of Gordiacea may encyst on vegetation and infect their host by mouth.

Many species of Gordiacea have been named without sufficient description to distinguish them. Identification must be based on details of the extremities and especially on the minute structure of the cuticula: its areolae, pore canals, furrows, tubercles, bristles.

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These studies were made under the direction of Dr. H. W. Manter whom the author wishes to thank for the loan of specimens and for many valuable suggestions. Specimens were also made available by Raymond Roberts and M. J. Harbaugh of the University of Nebraska.

METHODS AND MATERIALS

Adult GORDIACEA are collected from fresh-water habitats. Immature to adult specimens can be secured by dissection of the insect hosts (usually grasshoppers, crickets, or cockroaches). Individuals in a knot of more than 50 specimens of GORDIACEA, probably *Paragordius varius* (Leidy) found free-living in Kansas were still immature, demonstrating (as claimed by Camerano and Villot) that free-living forms are not necessarily mature.

For study of the extremities, a worm should be cut somewhat anterior to the cloacal aperture and posterior to the dark ring. For the study of the cuticula, cut small pieces from the middle third of the body. It is also desirable to secure an entire circumferential piece which, after some time in lacto-phenol solution, can be released from the underlying musculature. The cuticular structure often shows differentiation near the median lines. The technique of Müller (1927) and Heinze (1937) was used in making preparations of the cuticula. Study should be made of pieces in alcohol-diluted glycerin; lactophenol solution; water; Canada balsam and allowed to dry superficially. When possible histological sections of the cuticula are made since the surface examination alone is sometimes not enough, especially in the subfamily CHORDODINAE where minute details of areolae are important in specific diagnosis.

At least a combination of the cuticula and the posterior extremity must be used for specific characters. The shape of the posterior end alone is a weak character subject to many variations as verified by the author in females of *P. varius* and also in the postanal crescent of *G. robustus*. Drawings of the cuticula are almost necessary with descriptions. The author found that properly prepared pieces of cuticula lend themselves well to photomicrographs. Some indication was noted that polarized light might reveal added differentiation in the cuticula of some species.

TWO NEW RECORDS FROM MAN

Among the specimens studied was one adult female of *Paragordius varius* recovered, several years ago, from a child patient by Dr. G. R. Underwood of Lincoln, Nebraska. The specimen was passed in the feces in a living condition.

The second case is represented by an adult female of a *Paragordius* species (described below) given to the author by Dr. Ruy Gomes de Morais, Professor of Parasitology, National Medical School, Rio de Janeiro, Brazil. It was expelled alive from the urethra with severe pains suffered by the child host. The girl patient had frequently complained of pains in the inguinal region. The antecedents of this strange infection are not known, and it is the first, so far as the author can determine, reported from the urinary passages.

TAXONOMY

Today, the class GORDIACEA (= NEMATOMORPHA) is subdivided as follows:

Order NECTONEMATOIDEA Rauther, 1930 (all marine)
Order GORDIOIDEA Rauther, 1930
Family CHORDODIDAE May, 1919
Subfamily CHORDODINAE Heinze, 1935
Genus *Chordodes* Creplin, 1847
" *Euchordodes* Heinze, 1937
Subfamily CHORDODIOLINAE Heinze, 1935
Genus *Chordodiolus* Heinze, 1935
Subfamily PARAGORDIINAE Heinze, 1935
Genus *Paragordius* Camerano, 1897
Subfamily PARACHORDODINAE Heinze, 1935
Genus *Parachordodes* Camerano, 1897
" *Gordionus* Müller, 1927
" *Beatogordius* Heinze, 1934
" *Paragordionus* Heinze, 1935
Family GORDIIDAE May, 1919
Genus *Gordius* Linnaeus, 1766

Müller (1927) and Heinze (1934, 1935, 1937) have split the genus *Parachordodes* into a number of genera largely on the basis of the cuticula structure. The author believes that the genus *Chordodes* can well be divided into three genera based on the criteria used by Müller and Heinze for the genus *Parachordodes*. The following revision is proposed:

Genus *Chordodes* (Creplin, 1847) emend.

Posterior extremity of male not bilobed, with only comparatively shallow groove on ventral surface extending from cloaca to distal end. Posterior end of female usually swollen, entire, with terminal cloacal aperture. Cuticula usually with two or more types of papillar areolae, very prominent, variably grouped, the highest ones often bearing crown or tuft of transparent, long and slender filaments. Above areolae, or among them, scattered tubercles, hyalin processes and other structures are present. In most species, at least in female, there is median ventral furrow in which papillar areolae are more numerous and very conspicuous.

The genus comprises usually large species with geographical distribution confined to tropical or equatorial regions.

Type species: Chordodes pilosus Möbius, 1855.

Genus *Neochordodes* n. g.
(*Chordodes* (Creplin, 1847) pro parte)

(Fig. 11)

Tropical CHORDODINAE of unusually large size; anterior extremity slender, cylindrical; head attenuated or not, with obtusely truncated or rounded tip. Body cylindrical; posterior end narrower than immediately preceding portion, slightly flattened dorso-ventrally, rounded in female. In most species male provided with shallow median, ventral cloacal groove. Cuticula with single type of areolae, usually ovoid or rounded-polygonal in outline, very low in comparison with those of *Chor-*

dodes. Interareolar furrows with or without hyalin processes, bristles, or other structures.

Type species: Neochordodes talensis (Camerano, 1897) n. comb.

Other species: N. annadalei (Camerano, 1908) n. comb.; *N. molukkanus* (Römer, 1896) n. comb.; *N. weberi* (Villot, 1891) n. comb.; *N. moraisi* n. sp. (described below).

This genus seems well characterized by the shape of the posterior end of the male and by the single type of areolae. It might be mistaken for *Gordionus* which also has a single type of areolae, but it differs by the unforked posterior end of the male as well as by the lack of interareolar pore canals or rows of bristles or tubercles which are present in *Gordionus*. *Neochordodes* has only a few, scattered hyalin processes and sparse hairs or bristles. Differences from other genera are well marked.

Genus *Pseudochordodes* n. g.

(*Chordodes* (Creplin, 1847) pro parte)

Tropical Chordodinae of medium size. Usual shape is like genus *Chordodes;* anterior end pointed; posterior, median, ventral groove of male very weak, posterior to cloacal aperture which is usually no more than 0.5 mm from distal end. Cuticula with two types of very characteristic areolae: (1) usually polygonal areolae, mostly 5- or 6-sided, higher and darker than second type, in pairs or groups of three, rarely four, sometimes isolated. Most areolae bear pore canal with sessile bristle in narrow furrow which separates them; (2) low areolae, light in color, variable in shape and occupying greater part of cuticula. Interareolar furrows free of tubercles, bristles or other structures.

Type species: Pseudochordodes pardalis (Camerano, 1893) n. comb. (Fig. 12).

Other species: P. bedriagae (Camerano, 1896) n. comb.; *P. dugesi* (Camerano, 1898) n. comb.; *P. manteri* n. sp. (described below), and (with some question) *P. gestri* (Camerano, 1904) n. comb.

This genus differs from *Chordodes* by the presence of pore canals between the large areolae; from *Paragordionus* (which has the same type of areolae) by the unforked posterior end of the male and by the absence of interareolar structures; from *Euchordodes* by the absence of stout interareolar bristles. From other genera differences are well marked.

The author is satisfied that the following synonyms are justified. *Paragordius flavescens* Linstow, 1906 and *P. diversolobatus* Heinze, 1935 (Fig. 10) are synonyms of *P. varius*. Variation of the posterior end of the female of this species is common and unless a combination of this character is made with the cuticular structure no specific diagnosis could be surely established. The drawings of the cuticula presented by Heinze and von Linstow correspond exactly with all specimens of *P. varius* studied by the author. The bright halo mentioned and figured by von Linstow is merely a question of microscope adjustment (focus). Montgomery's drawings were evidently made under small magnification which probably led the German authors to regard their specimens as different species. The species described by Camerano as *Gordius danielis* in 1894 is considered by the author to be synonym of *Gordius paranensis*

Camerano, 1892. *Gordius guatemalensis* v. Linstow, 1902, *Gordius willeyi* Camerano, 1899 and *Gordius californicus* Camerano, 1915 should be considered as synonyms of *Gordius robustus* Leidy, 1850 since the slight variations of the shape of the lobes and crescent do not seem to be enough to separate them as different species. In the opinion of the author, *Gordius villoti* Rosa, 1882 is not a tenable species and most specimens referred to it in Europe are *G. setiger* Schneider, 1866 while the American specimens are probably *G. robustus* Leidy, 1850. The redescription of the latter species given by May (1919) agrees with specimens studied by the author.

Pseudochordodes manteri n. sp.

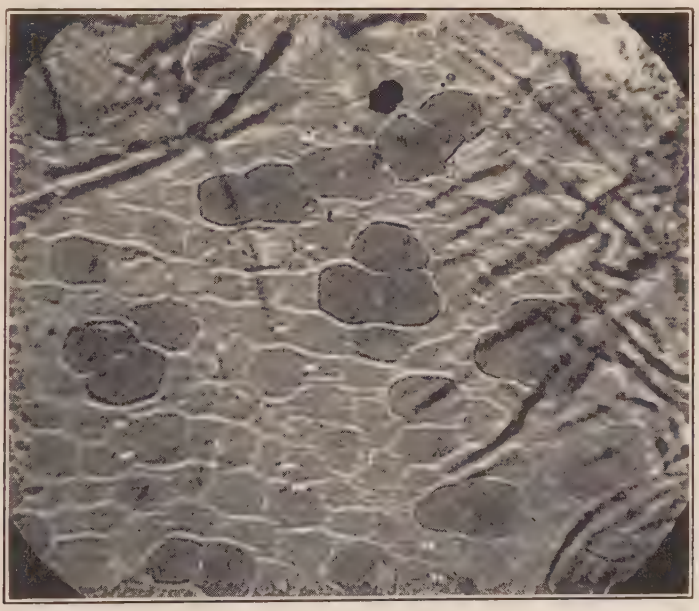
(Text-Fig. 1)

Dimensions: Length female, 160 mm; diameter near anterior end 0.354 mm; at midbody 0.9 mm, before swelling of posterior end 0.446 mm.

Form: Posterior end slightly pointed; sides of head almost parallel; mouth terminal. Dark ring, dorsal and ventral lines not visible. Posterior end with swelling near distal end, decreasing abruptly to more or less pointed posterior end. Cloaca terminal.

Color: Specimen (in alcohol) is dark; examined under light it has brownish color uniform over entire body.

Cuticula: With two types of areolae: (1) Large paired areolae, sometimes 3 or 4 together, scattered among second type and usually darker in color; rounded-elon-



TEXT-FIG. 1. Photomicrograph of the cuticula of *Pseudochordodes manteri* in lactophenol solution showing two types of areolae, interareolar furrows, pore canals and scattered paired tubercles (light spots) above some of the small areolae. The areolae are extended transversely. The dark fibrous shadows at right and upper left are remnants of body musculature (circa × 450).

gated or coarsely polygonal with rounded corners. These areolae higher than other type and larger diameter transverse to long axis of body. As characteristic for genus, they include pore canal between them in line that separates a pair or in center of 3 or 4 areolae. (2) Small, polygonal areolae usually four-cornered, sometimes triangular or pentagonal, extending crosswise to long axis of body and occupying most of cuticula, separated by narrow furrows showing no special structures. This type of areola is only type occurring in regions of dorsal and ventral lines where areolae are strongly polygonal, very irregular in size and form, extended longitudinally, and where interareolar furrows are not so visible as on sides of body. In region of ventral line, areolae form band about 0.15 mm wide. Characteristic for this species is presence of scattered paired tubercles above some of small areolae.

Areolae in general easily seen in lactophenol and in Canada balsam. External structure and paired tubercles are better visible in dried pieces of cuticula.

Locality: War Bonnet Canon, Nebraska, U. S. A. Specimen collected in freshwater.

Type: One female, deposited in the U. S. Nat. Mus., No. 36810.

This species seems to be similar to *Pseudochordodes pardalis* (Camerano, 1893) from Madagascar. It differs as follows:

P. manteri (Fig. 3)	*P. pardalis* (Fig. 12)
1. Large areolae without tubercles.	1. Large areolae with tubercles.
2. Small areolae bear one tubercle which often approximates another to appear paired.	2. Small areolae very sinuous, without tubercles.
3. Dorsal and ventral lines with small areolae only.	3. Dorsal and ventral lines with both types of areolae.

In addition, in *P. manteri* the small areolae and their furrows run transversely to the long axis of the body (Text-Fig. 2); the color is uniform, without spots; the shapes of the extremities are different. From *P. bedriagae* (Camerano, 1896) and *P. dugesi* (Camerano, 1898) differences are marked. The species is named in honor of Dr. H. W. Manter of the University of Nebraska.

Paragordius esavianus n. sp.

(Figs. 1–3)

Dimensions: Length female 211 mm, largest diameter 1 mm, diameter at dark ring about 0.39 mm, at level of trifurcation of lobes 0.55 mm.

Form: About 7 mm from posterior end (just posterior to atrium) abrupt narrowing of body from 0.693 to 0.492 mm. Anterior end pointed; dark ring distinctly visible so far anterior that with naked eye it seems at anterior end. With magnification, tip of head yellowish. Dorsal and ventral lines faintly visible. In region of dorsal and ventral lines and on sides of body are longitudinal, somewhat indistinct cuticular ridges visible in pieces of cuticula after releasing musculature. Posterior end (of female) trilobed, each lobe about 1.555 mm long; dorsal lobe slender, diameter at base 0.215 mm, in the middle 0.169 mm, near the tip 0.200 mm. This dorsal lobe has characteristic shape, narrower in middle, constriction near base. Lateral lobes 2 or 3 times wider than dorsal, diameter at base 0.539 mm, in middle 0.400 mm, near tip 0.231 mm, somewhat pointed and bearing internally pronounced excavation. They are very little divergent, practically in contact ventrally throughout entire length. On median and outer surfaces of lobes, in addition to spine-like cones of

cuticula, there are long, filiform hairs visible especially at the margins of the excavation.

Color: Brownish yellow with very marked dark ring. Extremities do not differ from body in color.

Cuticula (Fig. 3) : Entire surface of body evenly covered by numerous spine-like conical tubercles especially well seen in dried pieces of skin or in pieces in water under coverglass. In other liquids not well seen. When pieces of skin are superficially dried after being in diluted glycerin or lactophenol, cuticula shows formation of false areolae due to accumulation of liquid at bases of tubercles so that regular pseudo-areolar temporary formations are seen. In histological sections tubercles appear pointed with slightly rounded apex. They are straight and do not bear any refractive structure or hairs. Pore-canals and inter-tubercular bristles are apparently absent.

Locality: Cachoeira do Itapemirin, Estado do Espirito Santo, Brazil.

Host: This specimen, collected by Dr. Ruy Gomes de Morais, School of Medicine, Rio de Janeiro, Brazil, was expelled by girl through urethra, and seems to be first record of pseudo-parasitism by GORDIACEA of human urinary tract.

Type: One female, in collection of National Museum, Rio de Janeiro, Brazil.

This species differs markedly from others in the genus by the shape of the posterior end of the female and by the spine-like conical tubercles covering the cuticula. Very characteristic is the abrupt narrowing of the body in the region of the atrium not far from the posterior end. The species is named for E.S.A.V. (Escola Superior de Agricultura e Veterinaria, Viçosa, Minas Gerais, Brazil).

Neochordodes moraisi n. sp.

(Text-Fig. 2)

Dimensions: Length female 207 mm; diameter anteriorly 0.246 mm, in middle of body 2 mm, at narrow portion of posterior end of body 0.280 mm, short distance anterior to narrow portion of posterior end, 1.078 mm.

Form: Anterior end strongly attenuated, tip of head rounded. Body increases in thickness gradually toward middle and decreases again toward posterior end. Posterior end club-shaped. Cloacal aperture terminal and situated in middle of truncated distal end surrounded by yellowish area. Tip of head free of areolae but with small granulations. Dorsal and ventral lines and dark ring apparently absent.

Color: Yellowish brown, body covered throughout by very irregular brown spots which, joining here and there, give rise to larger spots. Anterior end slightly lighter and posterior end yellowish white around cloacal aperture.

Cuticula (Text-Fig. 2) : With one type of areolae usually rounded or ovoid, often with indentation on one side, with two, rarely three or more conical elevations more refractive. These usually paired above areolae. They may be found widely separated on areolar surface and sometimes near margins of two paired areolae giving false idea of pore-canals. Indentations of areolae usually well marked and reaching base of elevations. Greater diameter of areolae is transverse to long axis of body. Usually, areolae are isolated or in pairs but sometimes three or four together. Inter-areolar spaces wide, showing several furrows with some superficial ridge-like elevations. Scattered hyalin processes and fine tubercles in furrows. With low magnification, areolae appear arranged more or less in straight lines crossing long axis of body, and elevations as bright granules in pairs over areolae. In sections of cuticula, areolae show same height and elevations conical in shape with rather pointed tips. Here and there occur hyalin processes nearly same height as areolae. Appearance of cuticula in different liquids is rather constant. Areolae very distinct in diluted glycerine and in superficially dried pieces of cuticula.

Locality: E.S.A.V., Viçosa, Minas Gerais, Brazil.

Host: Collected by author from cockroach, *Blatta orientalis* Linn., in very poor condition, evidently severely affected by infection. (The striking and unusual development of the abdomen of the cockroach had attracted attention. After removal of the worm, one could scarcely believe that such a large worm had come from inside a cockroach.)

Type: One female, in collection of National Museum, Rio de Janeiro, Brazil.

TEXT-FIG. 2. Photomicrograph of the cuticula of *Neochordodes moraisi* in lactophenol solution showing the single type of areolae, and wide interareolar spaces with ridge-like elevations. Some of the areolae show two or three of conical elevations (circa ×450).

Areolae of one type with conical, paired or sometimes one or three prolongations do not occur in any other species of the genus.

This species is named in honor of Dr. Ruy Gomes de Morais.

SUMMARY

1. Two new cases of human infection with GORDIACEA are reported. One of these involved infection of the urinary passages.

2. The genus *Chordodes* is split into the genera *Chordodes, Neochordodes* and *Pseudochordodes*. The latter two are new.

3. *Gordius danielis* Camerano, 1894 is considered a synonym of *G. paranensis* Camerano, 1892. *G. guatemalensis* v. Linstow, 1902, *G. willeyi* Camerano, 1899 and *G. californicus* Camerano, 1915 are considered synonyms of *G. robustus* Leidy, 1850. *G. villoti* Rosa, 1882 is considered a synonym of *G. setiger* Schneider, 1866.

4. *Paragordius esavianus, Pseudochordodes manteri* and *Neochordodes moraisi* are described as new species.

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Explanation of Plate I, p. 222

Figs. 1–8 were drawn with the aid of a camera lucida.

Fig. 1. Posterior end of *Paragordius esavianus* (female), ventral view.

Fig. 2. Posterior end of *P. esavianus* (female), dorsal view.

Fig. 3. Spine-like conical tubercles of *P. esavianus* (female).

Fig. 4. Posterior end of *P. varius* (female), dorsal view.

Fig. 5. Group of areolae of *P. varius*, showing bright areas due to microscope adjustment (focus).

Fig. 6. Posterior end of *P. varius* (male), compressed, ventral view.

Figs. 7–9. Different groups of areolae of *P. varius*.

Fig. 10. Areolae of *P. diversolobatus* (after Heinze, 1935). Showing similarity to areolae of *P. varius*.

Fig. 11. Cuticula of *Neochordodes talensis* (after Camerano, 1897). Showing single type of areolae and granulations in interareolar furrows.

Fig. 12. Cuticula of *Pseudochordodes pardalis* (after Camerano, 1893). Showing large areolae with tubercles, and small areolae with sinuous irregular outlines.

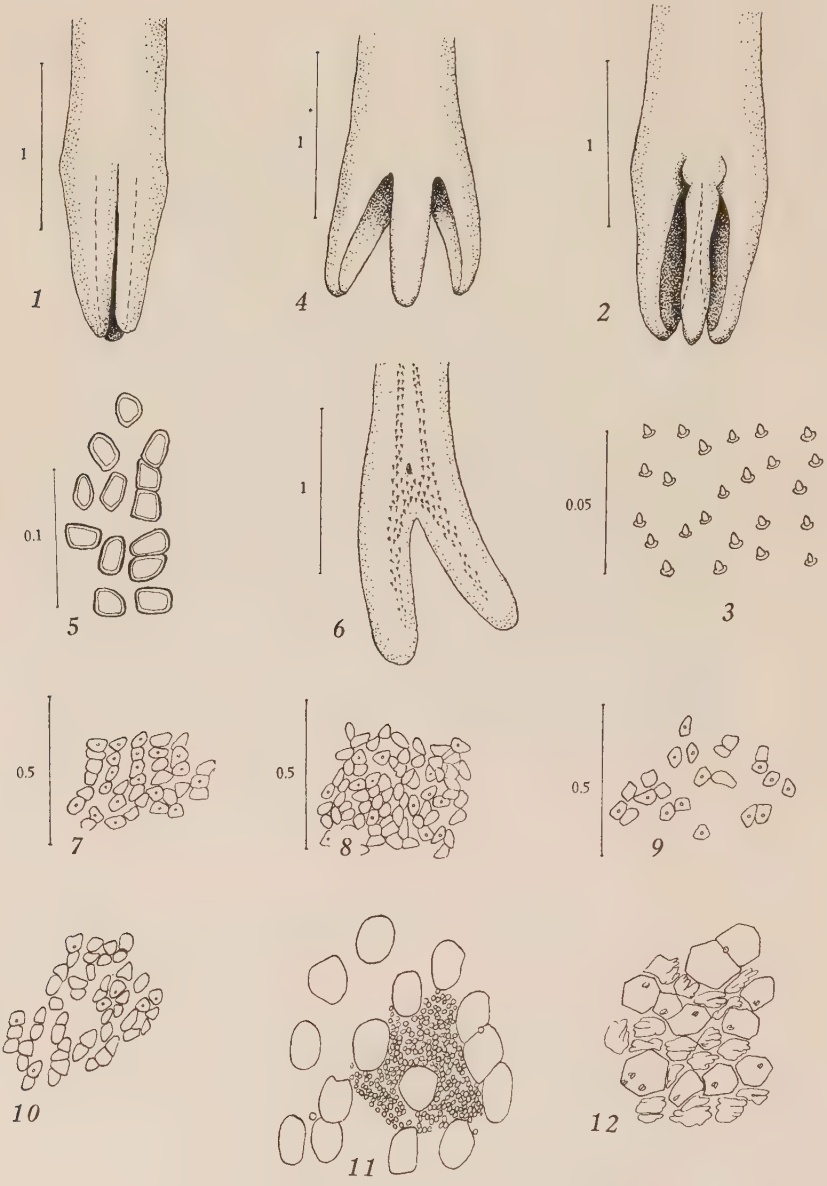

PLATE I

TRICHINELLA SPIRALIS. II. INCIDENCE OF INFECTION IN HOGS AND RATS IN THE NEW ORLEANS AREA

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Hinman (1) and Sawitz (2) through examination of autopsies have demonstrated that human infection with *Trichinella spiralis* occurs in the New Orleans area. Studying two square inches of diaphragm from each of 200 autopsies, Hinman found an incidence of 3.5 per cent. Sawitz, using larger samples and examining 400 autopsies, determined the incidence to be 6.0 per cent. In an earlier study Sawitz (3) detected an incidence of 5.0 per cent in 200 living persons by means of the intradermal test. In addition to man, rats, dogs and cats from this area have been examined. An incidence of 0.5 per cent was found in 200 rats (1), of 1.3 per cent in 300 dogs, and 10 per cent in 90 cats (2).

The present study was undertaken to determine the sources of human infection and to complete the studies on the incidence of *Trichinella spiralis* infection in its principal hosts in this area. This entailed the examination for *Trichinella* of both hogs and rats, and in the case of the latter, especially those rats which had had opportunity for association with hogs or pork.

SOURCE OF MATERIAL

Pork consumed in New Orleans comes from hogs that are raised in the South (Louisiana, Mississippi and Alabama), hogs that are raised on the outskirts of the city, and those that are raised in the Midwest. Southern hogs, including those raised near the city, are shipped in alive and are slaughtered locally. Midwestern hogs are usually received already slaughtered and dressed, the principal product being loins. Since no figures on the actual consumption of pork are available, the figures presented in Table 1 have been computed from municipal tax receipts.

TABLE 1.—*Hogs consumed in New Orleans as indicated by municipal tax receipts*

	1934	1935	1936	1937	Total
Southern hogs	21,816	20,156	30,708	36,285	108,965
Midwestern hogs ...	90,552	58,494	84,850	78,391	312,287
Total	112,368	78,650	115,558	114,676	421,272

These figures based upon tax receipts are not complete, because there

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are several concerns which sell pork in New Orleans, but do not operate branch houses, and do not pay the tax. The number of these hogs may be estimated at 25,000 annually. Combining these figures with those presented in Table 1 it is seen that the pork supply of New Orleans consists of approximately four-fifths Midwestern hogs and one-fifth Southern hogs.

There is a difference in the food supply of these hogs. The hogs raised on the outskirts of the city are fed mostly upon garbage; those from other parts of the South are allowed to roam in the fields and uproot peanuts where they are present; and those from the Midwest are fed largely upon grain. It seems reasonable to assume, however, that all hogs receive some garbage, even if it is only the unused material from the family table. In the present study hogs were examined from the sources mentioned above.

During this study rats also were examined, special emphasis being placed on those frequenting local hog farms, the two local abattoirs, and two local rendering plants.

METHODS OF EXAMINATION OF MATERIAL

Pork samples of Southern hogs were obtained in the abattoirs immediately after the animals had been slaughtered. Large pieces of diaphragm were cut from the hogs. Midwestern pork samples were obtained from branch houses of firms operating branches in the city and from butchers handling pork of packers not operating New Orleans branches. All samples of Midwestern pork were cut from fresh, unfrozen loins.

The pork samples were prepared for examination by the artificial digestion technic. A solution of 1.0 per cent pepsin in 0.6 per cent hydrochloric acid was allowed to act upon 50-gram samples of the ground meat at a temperature of 38° C for 15 to 20 hours. During this time occasional stirring was done. The resulting material was strained through two layers of cheesecloth into a large funnel which was equipped with a short piece of rubber tubing having a screw clamp at the delivery end. The material was allowed to settle for 2 to 3 hours, during which time the liquid in the upper part of the funnel was lightly stirred once or twice. After this sedimentation period, about 30 ml. of the bottom material were drawn off into a Petri dish and examined. In addition, approximately 250 samples were examined by the compressor technic.

The rats studied were caught in guillotine-type traps on local hog farms, the two local abattoirs, and two local rendering plants. The diaphragms of these rats were removed and immersed in 50 per cent glycerin solution. After 24 hours they were transferred to undiluted glycerin and, after another 24 hours, compressed between two slides and examined.

RESULTS OF EXAMINATIONS

Five hundred sixteen Southern hogs were examined and found negative for *Trichinella*. Seven hundred ninety-two loins representing at least 399 Midwestern hogs were examined and also found negative. Fifty samples of pork sausage bought from butchers and probably made up of both Southern and Midwestern pork, were examined by artificial digestion with negative results. Thus examination of 915 hogs and 50 samples of pork sausage consumed in New Orleans revealed no presence of *Trichinella*.

Of 467 rats examined, two were found infected with *Trichinella*, an incidence of 0.4 per cent. One of the positive rats was trapped on one of the hog farms, the other in a rendering plant located near several hog farms. In none of 280 rats from the abattoirs was *Trichinella* infection detected.

DISCUSSION

Although the studies of Hinman and Sawitz indicate an incidence of 5 to 6 per cent *Trichinella* infection in the human population of the New Orleans area, an examination of pork as the source of infection has revealed no positives among 915 hogs sampled. Furthermore, 50 samples of pork sausage were also negative.

The failure to find any positives in 516 Southern hogs indicates that the percentage of infection in the hog population of this area is low. Calculated from the binomial distribution and Fisher's fiducial probability theory, the probability is only 0.025 that the percentage of infection in these hogs exceeds 0.73 per cent. A similar low incidence in the South was determined in 1881, by Deverson (4), who detected only 0.4 per cent positives among 5400 hogs and, in 1883, by Detmers (5) who found no positives in 4146 Southern-raised hogs.

The significance of the failure to find any infected hogs in 399 Midwestern hogs may be expressed statistically as a probability of only 0.025 that the percentage of infection in that group exceeds 0.95 per cent. After an examination of thousands of samples Hall (6) reported incidences of 1.5 and 4.8 per cent respectively for grain-fed and garbage-fed Midwestern hogs. Schwartz (7) reported incidences of 1.0 and 5.0 per cent for similar groups. Hood and Olson (8) found a 0.4 per cent incidence in 500 grain-fed Midwestern hogs.

The 0.4 per cent *Trichinella* infection in 467 rats examined approximates Hinman's results (1) of 0.5 per cent in 200 rats taken at random in New Orleans. These comparable figures in rats indicate no appreciable difference in *Trichinella* incidence in rats associated with hogs as compared with rats living in the metropolitan area of New Orleans.

Of special interest is the absence of positives in 280 rats taken from the abattoirs. Many of these rats probably had opportunity to feed

upon stored pork, and if trichinous pork were occasionally stored in the abattoirs, the incidence of *Trichinella* in these abattoir-rats should be greater than in rats not frequenting places of pork storage. Thus the absence of infection in these 280 rats is further evidence of the low incidence of *Trichinella* infection in Southern hogs.

While the actual source of the 5 to 6 per cent incidence in man has not been detected in this study, it may be assumed that this infection rate is maintained by an occasional infected hog which would be found only by examination of much larger numbers of hogs.

The incidence of *Trichinella* infection in its main hosts in the New Orleans area may be summarized from the present data and those of Sawitz (2) by using his epidemiological diagram:

		dogs 1.3%
man 5–6%	hogs 0–1%	rats 0.4%
		cats 10%

SUMMARY

During 1938 and 1939 the diaphragms of 516 Southern hogs and the loins of 399 Midwestern hogs were examined for *Trichinella* infection by the artificial digestion technic. None was found infected. Likewise, none of 50 samples of sausage from local butchers revealed infection. Two positives, or 0.4 per cent, were detected in 467 rats trapped in this area. These low incidences agree with the low infection rate in man in the New Orleans area, as contrasted with other areas of the United States.

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MESOCESTOIDES MANTERI N. SP. FROM A LYNX, WITH NOTES ON OTHER NORTH AMERICAN SPECIES OF *MESOCESTOIDES*

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There is considerable difference of opinion with respect to speciation in the genus *Mesocestoides*. Most writers, including Cameron (1925), Mueller (1928, 1930) and Joyeux and Baer (1932) recognize a considerable number of species parasitic in mammals, whereas Witenberg (1934) merges them all in the species *litteratus,* of which he recognizes three "forms" of doubtful status.

I have not had an opportunity to examine specimens of *Mesocestoides* from other parts of the world, so am not in a position to pass judgment on Witenberg's conclusions with respect to the forms which he studied from Palestine and Europe, but I have collected and examined specimens from various North American mammals which in my opinion undoubtedly represent three distinct species. Specimens obtained from an opossum are readily recognizable as Mueller's species, *latus,* originally reported from a skunk in Minnesota, and later from a kitten in Minnesota and an opossum in Illinois. Specimens from a raccoon from Lufkin, Texas, and some fragments from a dog and from a raccoon from Nebraska, reported by Coatney (1936) and kindly sent to me for study by Dr. H. W. Manter, correspond closely with Mueller's *variabilis* var. *maior,* reported from a skunk in California. Some specimens from a lynx in Nebraska, also kindly supplied for study by Dr. Manter, undoubtedly constitute a new species, for which the name *Mesocestoides manteri* is proposed.

Mesocestoides latus Mueller, 1928

Four specimens were obtained from an opossum at Houston, Texas, none possessing fully ripe segments, measuring 34.5, 76, 106, and 112 cm in length, thus exceeding the greatest length previously reported for this species (50 cm) by Mueller (1930). The 76 cm specimen had about 800 proglottids. Mature segments are about 1.4 to 1.8 mm wide and 0.45 to 0.7 mm long. The segments become approximately square at about the 600th segment. The terminal segments of the longest worm, which are almost fully ripe, are 3.4 mm long and 1.6 mm wide. This worm, as pointed out by Mueller, lacks calcareous corpuscles, and becomes very clear on prepared slides as compared with other species. Another characteristic feature, not mentioned by Mueller, is the sharply defined walls of

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the vagina; an enlarged portion of this organ serving as a seminal receptacle, lying just anterior to the cirrus pouch, persists even in the terminal segments of the worm, and is almost as conspicuous as the cirrus pouch. Still another peculiarity is the method of formation of the egg capsule (Fig. 1, g–k). This develops from a mass of dense tissue at the posterior end of the uterus, which differentiates into a carrot-shaped organ with thick fibrous walls and a broad lumen. The egg capsule develops from the middle portion of this organ, the anterior portion having a broad lumen through which eggs reach the capsule, the posterior part contracting into a tail-like process.

Mesocestoides variabilis Mueller, 1928

Mature specimens of this worm were obtained from two raccoons from Lufkin, Texas, and some young forms still in the tetrathyridium stage were found in a third raccoon from the same locality. Two complete worms were each approximately 20 cm in length with a diameter of 1.25 to 1.5 mm. The scoleces measured 320 and 380 μ in diameter, respectively, in one case clearly demarcated from the neck, in the other not. Primordia of genital organs first appear faintly several millimeters behind the head. Mature proglottids are 550 to 840 μ broad and 280 to 400 μ long. Ripe segments are 1.8 to 2.1 mm long and 1.25 to 1.75 wide, the junction with adjoining segments being 700 to 750 μ broad.

This species is most readily distinguished from *latus* by its small scolex, by the numerous calcareous corpuscles and denser muscles which make it much less transparent, and by the method of development of the egg capsule, which first forms like a bowl around a more or less spherical mass of eggs, which may persist, although a thick-walled sphincter-like structure, as in other *Mesocestoides,* develops anterior to the mass. The fragmentary specimens from a dog and a raccoon from Nebraska are tentatively referred to this species; only semi-ripe and ripe segments are available. The former show the method of formation of the egg capsule around an egg mass which seems to be characteristic of *variabilis.* The ripe segments, 3 to 3.6 mm long and 1.3 to 1.4 broad, are much larger than my Texas specimens of *variabilis,* and resemble those of *latus,* except that they have abundant calcareous corpuscles. The difference in size may be due to method of relaxation and fixation. My specimens were obtained from a raccoon's carcass that had been frozen.

Since the specimens here reported from Texas raccoons are intermediate in size between Mueller's typical *variabilis* from *Spilogale* and *Urocyon* and his variety *maior* from *Mephitis,* it is probable that, as Mueller (1928) suspected, the distinction between these two forms is not a valid one, and the varietal name *maior* should be dropped.

Mesocestoides manteri n. sp.
(Figs. 1 a–f, 2, 3)

Description: Length 0.9 to 1.6 cm, with maximum width, in ripe segments, of 1 to 1.25 mm; total number of segments only 25 to 55. Scolex 420–490 μ in diameter and 285 to 350 μ long, set off from neck inconspicuously or not at all. Suckers in two pairs, dorsal and ventral, 175–210 μ long and 155–180 μ wide, with strong muscles, slit entire length antero-posteriorly, and directed straight dorsad and ventrad or slightly forward. Neck 315 to 500 μ broad at junction with head, almost immediately becoming as wide or wider than head. Primordia of genital organs first seen faintly about 720 μ to 1 mm from anterior end; first evidence of segmentation at edges of worm about 1 to 1.5 mm from anterior end. Segments develop rapidly, becoming fully mature at 16th to 20th segment, including those in which the primordia are only faintly present. Only a few sexually mature segments before uterus begins developing. Mature segments from 700 μ wide and 485 μ long to 900 μ wide and 175 μ long. Usually only one ripe segment present, bell shaped; junction with preceding segment only 265 to 350 μ broad.

Testes about 30 to 45 in number, closely crowded inside longitudinal excretory canals and occupying all space not occupied by other organs, 4 or 5 situated outside canals on each side; spherical or slightly oval or uneven, from 48 by 48 μ to 50 by 60 μ in size. Cirrus pouch with posterior border at about middle of segment directed antero-dorsad, 88 to 110 μ in diameter, and 88 to 140 μ long as seen from flat surface of segment; contains very much coiled sperm duct, and persists even in ripe segments.

Ovaries oval or round, 90 to 110 μ long and 70 to 110 μ broad, close together and connected by a ventral bridge, from 0 to 20 μ apart; distance between outer borders of ovaries 165 to 245 μ. Vitelline glands smaller than ovaries, ventral to them, 65 by 70 μ to 88 by 88 μ, 65 to 95 μ between them; distance between outer borders 200 to 265 μ. Vagina only moderately convoluted, passing around cirrus pouch on irregularly alternating sides, and opening just anterior to cirrus. Uterus appears first a few segments after sexual maturity is reached, bending around cirrus pouch and ending short of anterior border of segment. A few segments farther back it begins to expand and to fill with eggs, quickly forming a series of saclike bodies, one large one anterior to cirrus. Meanwhile posterior portion becomes surrounded by a mass of nuclei, and elongates and enlarges into a gourd-shaped body with a narrow lumen somewhat enlarged posteriorly, and thick striated walls, especially dense in the goose-neck region. At this stage practically no remnant of uterus visible posterior to gourd-shaped structure. Egg capsule develops from posterior portion of lumen leaving no caudal appendage (Fig. 1 a–f). In last 3 or 4 segments caudal appendage grows out anew in characteristic s-shaped form, reaching a length of 350 to 450 μ. Egg capsule slightly longer than broad, 350 by 320 μ to 395 by 350 μ. Egg mass 245 to 275 μ broad and 220 to 260 μ long, truncated posteriorly; wall of capsule thickened posteriorly where tail-like appendage forms.

Host: Lynx (*Lynx rufus*).
Habitat: Small intestine.
Locality: Nebraska, U. S. A.
Type: U. S. Nat. Mus. Helm. Coll. No. 44941.

Mesocestoides manteri is smaller than any other described forms of the genus except *M. bassarisci* MacCallum, 1921. The latter species is inadequately described, and may possibly be identical with the species here reported, but *bassarisci* is said to have the testes confined between the lateral canals, and to have the mature segments indistinctly divided from each other.

Aside from its small size and small number of segments, this species

FIG. 1. Development of egg capsule from posterior part of uterus in *Mesocestoides manteri* (a–f) and *Mesocestoides latus* (g–k). Mass of eggs shown in *a* sometimes missing.

FIG. 2. *Mesocestoides manteri,* entire specimen.

FIG. 3. *Mesocestoides manteri,* ripe segment.

FIG. 4. *Mesocestoides variabilis* from raccoon, ripe segment, drawn to same scale as Fig. 3.

is distinguished by its relatively broad head and neck, the bell-shaped ripe segments, and the method of formation of the egg capsule and of the long, looped caudal appendage posterior to it (cf. Fig. 1 a–f and Fig. 1 g–k).

SUMMARY

A dwarf species of *Mesocestoides, M. manteri* n. sp., is described from a lynx in Nebraska. *M. latus* is reported from an opossum in Texas, and *M. variabilis* from a raccoon in Texas and from a raccoon and dog in Nebraska. Specific differences in method of formation of the egg capsule, and caudal appendage of the capsule, are pointed out.

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A STUDY OF TEMPERATURE EFFECTS ON GREGARINES OF *TENEBRIO MOLITOR* LARVAE*

SISTER MARY MURIEL MACDOUGALL

Gregarines in mealworms have been the objects of many investigations—Pfeffer (1910), Mühl (1921), Daniels (1938), and others—but these studies have been confined chiefly to the morphology or development of various species and have not been concerned with the effects of any physico-chemical variable on these parasites. Von Brand (unpublished) used temperature as a means of defaunating *Tenebrio molitor* from their gregarines. Reyer (1937), referring to von Brand's investigations, assumes that high temperature merely hastened the encystment of the parasites and did not kill them. It seemed of interest to study this mechanism of eliminating the parasites more intensively and to extend the investigations also to the spore stages.

MATERIALS AND METHODS

The gregarines used in this study were obtained from the intestines of mealworms and comprised the three species described and illustrated in the papers of Mühl (1921), and Daniels (1938), namely, *Gregarina steini, Gregarina cuneata,* and *Gregarina polymorpha.* The predominant form was *Gregarina polymorpha;* and since the reaction to temperature was apparently quite similar in all three species, it was not deemed necessary to keep separate records for each species. The infection rate of the mealworms used ranged in the different batches from 66 to 99 per cent. In examining the hosts for parasites, the entire alimentary canal of the animal was removed, crushed on a slide in 0.65 per cent saline solution, and observed under the microscope. For routine examination these fresh slides proved entirely satisfactory. In some instances, however, the parasites were stained with borax-carmine.

In conducting the defaunation experiments by the incubation method the mealworms were placed in sterile culture dishes and put in an incubator at 37.5 $\pm$ 1° C for varying periods of time. A small piece of dampened paper towelling added to each dish furnished sufficient moisture. At the close of the incubation period the hosts were transferred to a sterile culture dish and kept at room temperature until dissected. In carrying out experiments in which it was necessary to maintain higher

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temperatures for periods of 15 or 20 minutes, the procedure was somewhat changed. It was deemed unsatisfactory to place the hosts in air at higher temperatures, since for the short periods wanted there was no guarantee that the heat would penetrate the worms. Therefore, a simple water bath was constructed and used for these experiments. The mealworms were immersed in water in a 200-ml Erlenmeyer flask, which was closed with a one-hole stopper into which a thermometer had been inserted. This thermometer registered the temperature of the water which surrounded the mealworms. The flask was fastened to an iron stand and set into a water bath heated to the desired temperature. It was found convenient to use this same method in testing the viability of the gregarine spores in infected bran.

EXPERIMENTAL DATA

The first step was to attempt defaunation experiments on the host larvae by means of the incubation method as von Brand had done. The objective was to free the mealworms from their parasites without causing injury to these hosts. Fig. 1 gives a graphic representation of all the results obtained when ten different batches of mealworms were incubated at 37.5° C for periods ranging from one to six days. Of the 541 experimental animals incubated 51 died, 75 pupated, and 415 were examined. In the graph the percentage of parasite-free hosts is plotted against the incubation period as the abscissae. This curve shows that an incubation period of six days is sufficient to completely free the mealworms from their parasites.

Tenebrio larvae were then submitted to higher temperatures for shorter periods of time, and moist heat was used instead of dry heat. This difference affects, of course, only the hosts, since the gregarines in the intestine live always in moist surroundings. The heat applied to them in the incubation experiments must also be regarded as moist heat. The control group used in this test showed an infection of 85 per cent. Fifty experimental animals were kept immersed in water at 40° C for 15 minutes. The temperature was maintained by means of the water bath previously described. When the hosts first came in contact with the warm water they became unusually active, and twisted and squirmed energetically in an attempt to crawl up the side of the flask and the stem of the thermometer. This pronounced activity gradually diminished and after about 10 minutes only a few of the worms showed any motility and those few were very lethargic. At the end of 15 minutes all motility ceased and the larvae were removed from the flask and placed in a dry, sterile, culture dish. The entire batch of worms was inactivated. By the next morning, however, most of the larvae had revived, although many of them were more lethargic in their movements than usual. When

examined, it was found that the infection rate had dropped from 85 to 17 per cent. The marked reaction which the hosts showed to the moist heat in this experiment led to the assumption that they would not be able to withstand a temperature much higher than that which killed their parasites. In order to determine the thermal death-point of the hosts under these conditions, 50 worms were immersed in water at 42.5° to

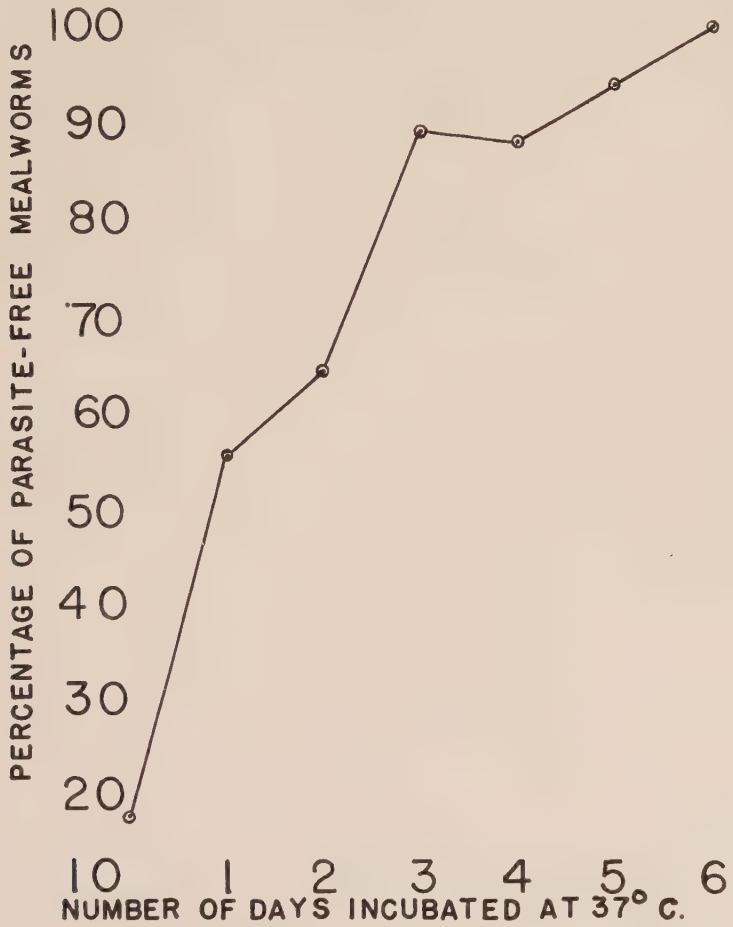

FIG. 1. Defaunation of mealworms kept at 37.5° C.

43.5° C and this temperature maintained for a 15-minute period. Inactivation of the larvae was observed shortly after immersion, and not one of these hosts revived. In other words, the thermal death-point of the gregarine trophozoite, when death is achieved by a short exposure to high temperatures, is only about 3° lower than that of their hosts.

The experiments mentioned so far show that a temperature of 40° C is lethal to the gregarines but they are not sufficient to decide whether or

not Reyer's hypothesis concerning the action of a temperature of 37° C is correct. The decisive test had to be preceded by reinfection experiments.

One hundred and thirty-five mealworms were first defaunated by incubation for one week at 37.5° C, and after removal from the incubator kept at room temperature and fed bran containing the spores of the gregarines for periods of 7, 10, and 14 days. The spore-containing bran was obtained from the jar in which the stock culture of infected *Tenebrio* larvae was kept. The average reinfection which took place in the three feeding periods was 91 per cent. In each lot examined in this series very small gregarines were present which indicated the expected "new infection," but together with them large ones appeared in some of the larvae, which might be interpreted as an "old infection." In order to account for these large gregarines another test was made. This time 29 defaunated larvae were put into spore-infected bran for only three days. Examination of the hosts at the close of the three day feeding period showed 25 parasite-free larvae and only one infected larva. (Three of the lot were dead.) The one positive finding revealed a very sparse infection of only small gregarines. It may be supposed that actually more mealworms were already infected, but that the parasites were still in the intracellular stage and thus escaped detection. Therefore, it seems likely that the large gregarines noted in the earlier reinfection experiments were due to the fact that some of the larvae may eat spores on the first day that they are in the infected bran and so become parasitized earlier than some others in the same lot that may not have come in contact with the spores until several days later.

The fact that mealworms were so easily reinfected allows a further, and, it is believed, a decisive test concerning Reyer's hypothesis. Fifty worms, defaunated in the usual manner, were put into spore-infected bran for one week. They were then removed from the bran and 20 of the lot were examined. Seventeen of these showed a heavy infection of both large and small gregarines, two showed a heavy infection of only small gregarines, one was negative. The remaining 30 of this lot were not examined but were put into a sterile culture dish and incubated at 37.5° C for a period of four days. If a temperature of 37.5° C does not kill the gregarines but merely hastens the life cycle, as Reyer assumed, the incubation period of four days with newly reinfected larvae should not eliminate the parasites, but merely allow the young infections to grow to the size of the old infections. However, when this lot was examined, 29 were found to be negative; one had died. The legitimate conclusion is that the gregarine trophozoites had actually been killed.

These experiments conducted on the trophozoite stage of the gregarines were followed by a series of experiments designed to test the viability

of the spore stage at various temperatures. The criterion of spore viability was to be the number of infections observed in larvae fed upon the spore-containing bran after it had been subjected to varying temperatures. Bran taken from the infected stock culture, and containing a quantity of the granular, sandy excreta in which the gregarine spores occur, was placed in a 200-ml Erlenmeyer flask. The flask was equipped with a thermometer, the bulb of which was placed in the center of the bran. The flask was immersed in a water bath and the desired temperatures maintained for periods of 15 or 20 minutes after the bran had reached the intended temperature. At the end of this time the bran was brought to room temperature and transferred to a sterile culture dish. Mealworms which had been previously defaunated by the incubation method were put into this bran and kept at room temperature for a period of one week, at the end of which time they were examined for the presence of gregarines. The results of this series are summarized in Table 1. Temperatures of 49°–50° C and 60°–64° C did not kill the

TABLE 1.—*Spore viability tested by dry heat*

Temperature to which spores were heated	Number of hosts fed on test bran for 7 days	Remarks	Number of hosts showing gregarine infection	Number of non-infected meal-worms	Percentage of hosts infected by viable spores
49–50° C–15 min.	54	2 pupated; 3 dead; 49 exam.	39	10	80%
60–64° C–15 min.	39	10 dead; 29 exam.	22	7	76%
68–70° C–20 min.	50	50 exam.	1	49	1%
95–96° C–20 min.	56	10 dead; 46 exam.	4	42	9%

spores, but they were destroyed if exposed for 20 minutes to 68°–70° C.

Dry spore-infected bran was also submitted to incubation at 37.5° C for periods of 50 and 90 days, but the spores were still viable as 60 per cent of the parasite-free mealworms fed on this bran became infected in the first instance, and 73 per cent in the second.

In order to decide whether moist heat would prove more detrimental to the spores than dry heat, infected bran was placed in a flask—in the same manner as described above—and covered with water heated to the same temperature as that of the water bath in which the flask was placed. This temperature varied in each test and was maintained for periods of 15 or 20 minutes. After heating, the bran was removed from the flask, spread out in a pan and allowed to dry in air. When thoroughly dry, the bran was ground in a mortar in order to break up the large clumps formed when the bran was wet. This bran was then put in a sterile culture dish and mealworms which had been previously defaunated were put in the dish to feed upon the bran for a period of one week. This series is summarized in Table 2. A temperature of 39° – 40° C for 20 minutes

TABLE 2.—*Spore viability tested by moist heat*

Temperature to which spores were heated	Number of hosts fed on test bran for 7 days	Remarks	Number of hosts showing gregarine infection	Number of non-infected mealworms	Percentage of hosts infected by viable spores
39–40° C–20 min.	28	1 dead; 27 exam.	20	7	74%
54–55° C–20 min.	40	2 dead; 38 exam.	none	38	–
63–64° C–20 min.	50	5 dead; 45 exam.	none	45	–
48–50° C–15 min.	43	2 pupae; 33 exam. 10 dead.	none	33	–

had no detrimental effect on the spores but temperatures of 48° to 50° C and above killed the spores.

DISCUSSION

Cleveland (1924) was able to completely defaunate termites (without injuring these hosts) by incubating them at 36° C for a period of 24 hours. Von Brand (unpublished) sterilized *Tenebrio* larvae by incubating them at 37° C for a period of five days. In this study, complete defaunation was accomplished by a six-day incubation period at 37.5° C. In contrast to Reyer's opinion, these experiments prove that this temperature actually kills the trophozoite stage of the gregarines and does not merely accomplish defaunation by causing the vegetative stage to encyst. The fact that gregarine trophozoites disappear from the mealworms after the latter have been immersed for a short time into water of 40° C is doubtless due to a lethal influence of this temperature. The mealworms become, as mentioned above, immotile, but recover after a certain time. The suspicion that the gregarines were not killed by this temperature, but by asphyxiation which might result from a curtailment of breathing activities of the hosts during the period of immobility, was disproved by von Brand. He (personal communication) subjected mealworms to anaerobic conditions lasting four hours, during which time they ceased all movements. After recovery they showed only an insignificant drop in infection rates as compared to the control group.

The experiments testing spore viability bring out the fact that the spores could be killed by moist heat at 48°–50° C for 15 minutes, or by dry heat at 68°–70° C for 15 minutes. In comparing these results with those of other investigators who made similar studies on the viability of cysts of intestinal protozoa, we find that there is considerable variation in viability even among parasites living in the same environment. Boeck (1921) made extensive studies on the resistance of human intestinal cysts to moist heat with five minutes exposure at 48°–52° C. When exposed to temperatures higher than 52° C, the number of dead *Endamoeba histolytica* cysts increased with each increase of temperature until all of them were dead at the thermal death-point of 68° C. *Endamoeba*

coli cysts displayed a high degree of resistance from 52° to 64° C, and their thermal death-point was not reached until they had been exposed for 5 minutes at 76° C. The cysts of *Iodamoeba bütschlii* were less resistant, as large numbers died after being exposed to temperatures above 56° C; and the thermal death-point was reported to be 64° C for five minutes. *Endolimax nana* cysts also had a thermal death-point of 64° C, as did *Giardia intestinalis* cysts. *Chilomastix mesnili* cysts proved to be almost as resistant to heat as were those of *Endamoeba coli,* as their thermal death-point was reached after a 5-minute exposure to 72° C.

Reinhardt and Becker (1933) subjected the cysts of *Eimeria miyairii* to moist heat maintained by means of an electrically controlled water bath. They found that the unsporulated oöcysts were killed in 15 seconds at a temperature of 53° C and in 24 hours at a temperature of 41° C.

Ellis (1938) tried the effect of dry heat exposure (incubation method) on sporulated and unsporulated oöcysts of *Eimeria tenella.* Sporulated oöcysts became non-viable after one day exposure to a temperature of 39° C. At this same temperature unsporulated oöcysts did not sporulate. Neither did sporulation occur when the cysts were exposed to temperatures as low as 4.4° C or as high as 37° C for 48 hours; however, the viability of the cysts was retained as sporulation promptly occurred when these cysts were placed at 21.1° C.

SUMMARY

1. An incubation period of six days at 37.5° C is sufficient to completely defaunate mealworms from their parasitic gregarines and this temperature actually kills the gregarine trophozoites and does not merely hasten their encystment.

2. The thermal death-point of the trophozoite stage is 40° C when exposed to moist heat for 15 minutes.

3. The thermal death-point of this parasite is only 2° to 3° lower than the thermal death-point of its host.

4. The defaunated hosts may be reinfected by feeding them bran containing gregarine spores.

5. Spore viability is destroyed when gregarine spores are exposed to dry heat at a temperature of 68°–70° C for 20 minutes; or when they are exposed to moist heat at a temperature of 48°–50° C for 15 minutes.

6. Spore viability is not affected by an incubation period of ninety days dry heat at 37.5° C.

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A NEW SPIRUROID NEMATODE, *THELAZIA BUTEONIS*, FROM SWAINSON'S HAWK*

KARL E. HERDE

In April 1939 a Swainson's hawk, *Buteo swainsoni* Bonaparte, 1838, collected near Tahlequah, Oklahoma, was found to contain in the orbit of one eye a male and six female specimens of an apparently new thelaziid nematode. The worms were preserved in 10 per cent formalin. Later they were washed in water, partially dehydrated in alcohol, and transferred to glycerin for study.

Thelazia buteonis n. sp.

(Figs. 1–4)

Description: Body slender, transparent, and slightly milky or colorless in life. Cuticle marked by numerous (74–86 per mm of body length) annular constrictions which are very distinct anteriorly but gradually fade out toward the rear and become unrecognizable at a level slightly in front of the anus—optical margins of anterior two-thirds of body slightly serrate in appearance because of these annulations. Mouth and buccal cavity roughly circular in en face view. Cephalic papillae, present in the number and arrangement characteristic of the genus, include, besides the prominent amphids, six minute internals, medium-sized dorsodorsals and ventroventrals, and large laterodorsals and lateroventrals. Nerve ring prominent, near middle of esophagus. Deirids somewhat behind nerve ring.

Female: Measurements from 5 mature preserved specimens under cover glass pressure. Length 13.0 (12.1–13.9) mm, width near middle of body 605 (471–648) μ. Buccal cavity—length 35 (28–41) μ, width 50 (41–55) μ. Esophagus—length 562 (506–615) μ, greatest width 84 (80–87) μ. Distance from anterior end—nerve ring 286 (275–300) μ, vulva 575 (522–647) μ, deirids 391 (388–413) μ. Tail broadly conical with a rounded tip; 210 (187–233) μ long. Vulva near level of posterior end of esophagus, being sometimes slightly in front of and sometimes slightly back of this point.

Male: The single preserved specimen was measured under cover glass pressure. Length 11.1 mm, width near middle of body 573 μ. Buccal cavity 27 μ long and 45 μ wide. Esophagus 487 × 87 μ. Nerve ring 258 μ from front end. Tail broadly rounded at tip, 173 μ long. Spicules—right 204 × 36 μ, stout, heavily chitinized, and with a rugged surface; left 1658 × 9 μ, delicate and acicular. Caudal papillae include 8 left, 9 right, and 1 median preanals; 1 right para-anal; and 7 pairs of rather symmetrically arranged postanals.

Host: Buteo swainsoni Bonaparte, 1838.

Habitat: Orbit of eye.

Locality: Tahlequah, Cherokee County, Oklahoma.

Specimens: Four females and one male in alcohol, in U. S. Nat. Mus. Helm. Coll., No. 36804 (cotypes).

The genus *Thelazia* Bosc, 1819, contains 31 previously known species all of which appear normally to be parasitic about the eyes of birds or mammals. The new species was studied in comparison with descriptive

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* A contribution from Northeastern State College (Tahlequah, Oklahoma) and (No. 95) the Zoological Laboratory, Oklahoma Agricultural and Mechanical College; prepared under the direction of R. Chester Hughes.

data on all of the others. The writer's sources of information were the compilatory accounts of Cram (1927) and Price (1931) for the older forms and the respective original descriptions of Baylis (1920 and 1934b), Dubinina (1937), Ershov (1928), Hsü (1933 and 1935), Oserskaja (1931), Price (1930), Railliet and Henry (1910a and 1910b), Sandground (1933), Schuurmans Stekhoven (1937), Smit and Noto-Soediro (1930), and Wehr (1930) for the several forms whose specific names first appear in them.

The 20 previously described avian species of *Thelazia* differ from *T. buteonis* n. sp. as severally indicated below:

T. annamensis Sandground, 1933, *T. campanulata* (Molin, 1858) Railliet and Henry, 1910, *T. cholodkowskii* Skrjabin, 1922, *T. cirrura* (Leidy, 1886) Railliet, 1916, *T. dacelonis* (Breinl, 1913) Travassos, 1918, *T. digitata* Travassos, 1918, *T. digiticaudata* Schuurmans Stekhoven, 1937, *T. longicauda* Sandground, 1933, *T. lutzi* Travassos, 1918, *T. nyctardeae* Dubinina, 1937, *T. stereura* (Rudolphi, 1819) Railliet and Henry, 1910, and *T. tonkinensis* Hsü, 1935, all have the vulva in the female located cephalad of the anterior end of the intestine by at least one-fourth of the length of the esophagus;

T. anolabiata (Molin, 1859) Railliet and Henry, 1910, has finger-like processes that extend into the buccal cavity from the rim of the mouth;

T. aquilina Baylis, 1934b, is considerably larger and has only 4 pairs of postanal papillae in the male; in

T. chui Hsü, 1935, described from a single male specimen, the body is considerably larger, the right spicule is much shorter in proportion both to the length of the body and the length of the left spicule, and a "gubernaculum-like" structure occurs near the cloaca; in

T. chungkingensis Hsü, 1933, the body is smaller in both sexes, the tail of the female is relatively shorter, and the male has fewer caudal papillae; in

T. dentifera Sandground, 1933, the tail is much shorter in both sexes, the male has fewer caudal papillae, and the left spicule is less than twice as long as the right;

T. depressa Baylis, 1920, is considerably larger and has the tail in both sexes terminating in a peculiar digitiform process; in

T. papillosa (Molin, 1860) Railliet and Henry, 1910, the body is more slenderly elongate, the neck is provided with delicate cuticular spines, the tail of the female is more bluntly rounded, and the male has fewer caudal papillae; and in

T. philippinensis Wehr, 1930, the male has fewer postanal papillae, the length of the left spicule is greater in proportion to that of the right, and the tail of the female is relatively much shorter.

The 11 mammalian species differ from the new form as follows: in *T. alfortensis* Railliet and Henry, 1910a, *T. californiensis* Price, 1930, *T. erschowi* Oserskaja, 1931, *T. floresiana* Smit and Noto-Soediro, 1930, *T. gulosa* Railliet and Henry, 1910a, *T. lachrymalis* (Gurlt, 1831) Railliet and Henry, 1910, *T. leesei* Railliet and Henry, 1910a, and *T. rhodesii* (Desmarest, 1828) de Blainville, 1828, the vulva of the female is situated caudad of the anterior end of the intestine by a distance equal to at least one-third of the length of the esophagus;

T. callipaeda Railliet and Henry, 1910b, has only 2 pairs of postanal papillae in the male; and in

T. iheringi Travassos, 1918, and *T. skrjabini* Ershov, 1928, the males have more numerous preanal and fewer postanal caudal papillae.

Thelazia depressa was originally found in a mongoose but Baylis (1934a) regards it as being normally a parasite of vultures.

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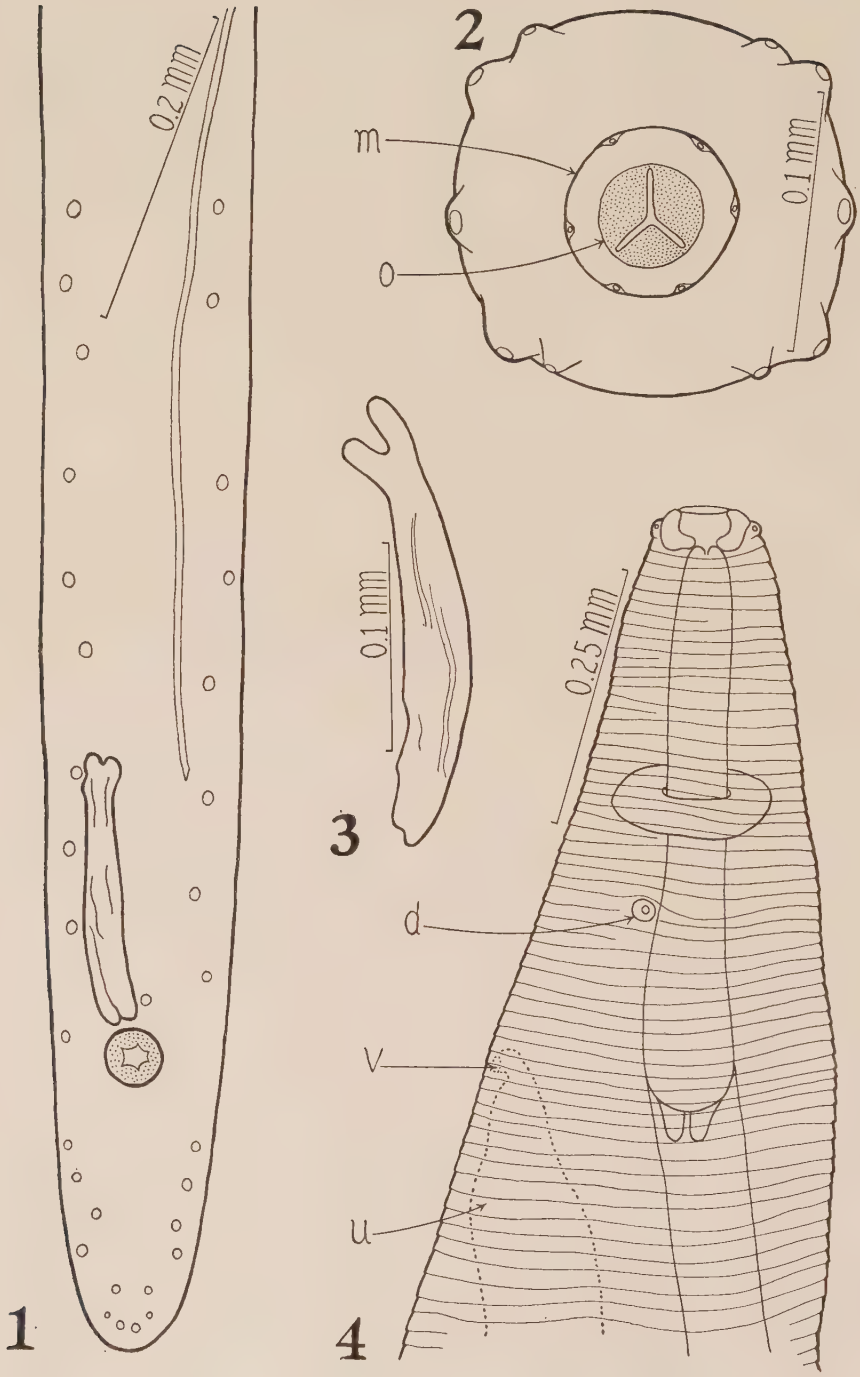

Thelazia buteonis n. sp. All figures made with the aid of a camera lucida.
d—deirid; m—rim of mouth; o—front end of esophagus; u—uterus; v—vulva.

FIG. 1. Tail of male. Ventral view. FIG. 2. Anterior end. En face view.
FIG. 3. Right spicule. FIG. 4. Anterior end of female. Sinistral view.

RESEARCH NOTES

OCCURRENCE IN THE HORSE OF TWO PARASITES OF CATTLE, *OSTERTAGIA OSTERTAGI* (STILES, 1892) AND *COOPERIA ONCOPHORA* (RAILLIET, 1898)

Unusual parasites, normally inhabiting the alimentary tract of ruminants, in particular cattle, were met with three times in the horse when a large number of horse stomachs and duodenums were examined for parasites.

Case 1 was a four-year-old horse. The stomach showed signs of a chronic hypertrophic gastritis with several circumscribed adenomatous changes in the mucosa, which were due to the presence of numerous *Trichostrongylus axei* (Cobbold, 1879), a parasite already known to occur in both horses and ruminants. In addition, two unfertilized females of the genus *Ostertagia* were found in the stomach, both apparently belonging to the species *O. ostertagi* (Stiles, 1892), the so-called medium stomach worm of the ox. (One of the females was somewhat injured, so that in this case the diagnosis of the species had to be made with some reservation.)

Case 2 was a fifteen-year-old horse that had died of chronic gastroenteritis. The stomach and the duodenum were markedly inflamed. A slight infection with *Trichostrongylus axei* was present in the stomach. Furthermore, 14 mature specimens (5 males and 9 females) of *Ostertagia ostertagi* (Stiles, 1892) and one mature male of *Cooperia oncophora* (Railliet, 1898), a parasite of the small intestine of the ox, were found in the duodenum, while a female of *O. ostertagi* occurred in the stomach.

Case 3 was a one-and-a-half-year-old filly coming from the same pasture as case 1. Its stomach also showed the characteristic changes due to an abundance of *Trichostrongylus axei.* In addition, 5 mature *Ostertagia ostertagi* were found, viz., 3 males and 2 females, 2 of which were in the stomach, the remainder in the duodenum.

So far as the authors are aware this is the first record from the horse of two parasites, otherwise so typical of ruminants, particularly cattle, as are *O. ostertagi* and *C. oncophora.* It may be added that the size of the specimens found in the horses was in all cases nearly the same as in their natural hosts. The male of *C. oncophora* measured about 6.5 mm, the males of *O. ostertagi* from 5.0 to 6.5 mm and the females of *O. ostertagi* from 5.5 to 8.0 mm. Almost all females of *O. ostertagi* in cases 2 and 3, where males also were present, contained typical eggs in the morula stage.—Hans Roth and N. O. Christensen, *Department of Special Pathology, Royal Veterinary and Agricultural College, Copenhagen (Director: Professor H. C. Bendixen).*

ORAL TRANSMISSION OF *PLASMODIUM CATHEMERIUM* BY MEANS OF TISSUES

Four years ago, an experiment was performed by the author in order to determine whether the exoerythrocytic stages of *Plasmodium cathemerium* in canaries could be transmitted by the oral route. The result was positive in one of four cases. Since an abrasion of the alimentary canal in the process of inoculation was a possible explanation of the positive result, this experiment was not reported at that time. The present report seems justified, however, because of the recent interest in the transmission of *Plasmodium* by the oral route (Shortt & Menon, 1940, J. Malar. Inst. Ind.; Beltran & Larenas, 1941, Rev. Inst. Salub. Enferm. Trop.; and Young, 1941, Pub. Health Rep. U. S. Pub. Health Serv.). The host, the species of *Plasmodium,* the use of tissue and the presence of exoerythrocytic stages in the inoculum makes the present experiment different from those previously reported.

Canary 627 died Nov. 18, 1937, from an infection with the wood thrush strain

of *Plasmodium cathemerium*. Its spleen, which showed both erythrocytic and exoerythrocytic stages, was macerated in citrated salt solution to make up a volume of 360 mgm. This mixture was administered into the crop of canary 637 by the use of a small catheter attached to a syringe. After an incubation period of 9 days, *Plasmodium* was observed in the peripheral blood of canary 637. The patent period of this infection lasted for five days and terminated in death. At autopsy, both erythrocytic and exoerythrocytic stages were observed in the lung, spleen, liver, bone marrow and brain of canary 637.

This experiment was repeated using three more canaries inoculated orally and a fourth, the control, inoculated intramuscularly. A portion of liver, the whole brain and the whole spleen of canary 636 (which died Nov. 28, 1937) were suspended separately in citrated salt solution. Each suspension made up a volume of about 400–460 mgm, and was administered orally into canaries 652, 653 and 654, respectively, using the same method as had been employed for canary 637. The control canary, 655, was inoculated intramuscularly with heart's blood from canary 636. This blood contained about 3,000 parasites per 10,000 erythrocytes; the tissues showed numerous erythrocytic and exoerythrocytic stages. The erythrocytes contained only uninucleate stages of *Plasmodium,* whereas the exoerythrocytic forms were represented by various stages from uninucleated stages to "ripe" schizonts apparently ready for, or in the process of, segmentation.

The control canary, 655, died from the infection 8 days after its inoculation. The other three birds survived and showed no parasites for a period of three months. After this period, one (canary 652) was killed. Its tissues were examined and found negative for malarial infection. The other two were reinoculated intravenously with the same strain of *P. cathemerium* and both died from the infection 13 days later, confirming the previous observation that they had not been infected by the oral route.

To summarize, tissues of canaries containing erythrocytic and exoerythrocytic stages of the wood thrush strain of *Plasmodium cathemerium* were administered by mouth to four canaries on two different occasions. One canary developed an infection. Parasites were observed in its peripheral blood 9 days after oral administration and exoerythrocytic stages were found in its tissues at autopsy 14 days after inoculation. The other three canaries remained uninfected: their inocula contained various exoerythrocytic stages and only mononuclear erythrocytic stages of development.—Fruma Wolfson, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

THE INTRAVENOUS INOCULATION OF SPOROZOITES OF *PLASMODIUM GALLINACEUM*

In 1936 Wolfson (J. Parasitol. **22**: 291–292) reported the first case of bird malaria, *Plasmodium praecox,* transmitted by intravenous injection of sporozoites in the canary. Three birds were inoculated with 150 mgm of saline-citrate solution, to which a small amount of sterile bovine serum was added. Each bird received sporozoites from about two infected *Culex pipiens.* In one of the canaries the result was negative; in the other two infections of different intensities appeared on the 10th day. Wolfson mentioned the unsuccessful experiments of previous workers.

In 1937, Warren and Coggeshall (Am. J. Hyg. **26**: 1–10) reported the intravenous inoculation of canaries with sporozoites of *P. cathemerium,* in saline solution, from infected salivary glands of *C. pipiens;* they said they had used this method for several months with "continuous success."

In this paper we report the intravenous injection of sporozoites of *P. gallinaceum* into two Leghorn chickens, each more than four months old.

Infected salivary glands of *Aedes aegypti* were crushed in a drop of Locke's solution, and suspended in the same fluid. The injection was performed in a wing vein. In order to insure that no sporozoites were accidentally injected outside the blood vessel, the syringe was charged without needle, a small air bubble was allowed

to enter the tip of the syringe, which was then carefully cleaned. A clean needle was inserted and no pressure exerted until it was ascertained that the needle was in the vein; the passage of the air bubble inside the blood vessel proved that the needle was correctly placed.

In the first chicken (Ga 168) the animal received sporozoites from the salivary glands of one mosquito. On the 6th day after the inoculation the blood was still negative, but on the 10th day parasites were found in the circulating blood in the proportion of 1,400 parasites per 10,000 erythrocytes.

In the second chicken (Ga 184) the animal received sporozoites from the salivary glands of about 1.5 mosquitoes. Parasites were found in the circulating blood on the 11th day after the injection, when their number was 9 per 10,000 erythrocytes.

The prepatent period after the injection of sporozoites intravenously seems to be the same as that observed after natural or artificial inoculation by subcutaneous or intramuscular routes. Our records (eight cases) vary from seven to eleven days. This similarity in the period of incubation after inoculation with sporozoites by different routes, was observed by Warren and Coggeshall in their studies with *P. cathemerium.*

The possibility of causing infections by intravenous injections of sporozoites is interesting, because of the gap existing in our knowledge of the primary stages of malarial infections naturally caused by mosquitoes. It is desirable to establish if all the species of plasmodia have or have not the possibility of being transmitted by intravenous injection of sporozoites.

The studies of James and associates (1927, Tr. Royal Soc. Trop. Med. and Hyg. **21**: 233–236) show that human malaria can be induced by intravenous inoculation of sporozoites. The papers of Wolfson, and Warren and Coggeshall, and our experiments here reported, show that the transmission of avian malaria by intravenous injection of sporozoites is possible in the canary with *Plasmodium praecox* and *P. cathemerium,* and in the chicken with *P. gallinaceum.*—Enrique Beltran and Luis Vargas, *Instituto de Salubridad y Enfermedades Tropicales, México, D. F.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Seventeenth Annual Meeting, Dallas, Texas

December 29 to 31, 1941

Minutes of the Seventeenth Annual Business Meeting

The seventeenth annual business meeting of the Society was held at Hotel Baker on December 30, 1941, following the annual Parasitologists' Luncheon, which was attended by 95 members and guests. The meeting was called to order at 1:20 PM by President James E. Ackert who introduced the officers of the Society and the five former Presidents who were present, Drs. Henry B. Ward, W. W. Cort, W. A. Riley, H. W. Stunkard and D. H. Wenrich.

I. Reports of Officers

1. The report of the Secretary was presented. As of December 10, 1941, there were 447 members in good standing and 65 who were delinquent in dues for from one to three years, making a total membership roll of 512. Thirty-eight new members were elected during 1941. The deaths of the following members occurred during the past year:

John E. Guberlet, Professor of Zoology at the University of Washington, December 30, 1940, at Seattle, Washington.

Charles Wardell Stiles, former President of the Society and Honorary Life Member, January 24, 1941, at Baltimore, Maryland.

Donald Cameron, of Ottawa, Canada, was killed in service with the Royal Canadian Air Force, June, 1941, at Malta.

The Secretary also reported the following actions of the Council at its meeting held on December 28, 1941:

(1) Acceptance of the recommendation of the Secretary that, in the future, simultaneous sessions be scheduled, if necessary, to fit the program into a three-day meeting period.

(2) Adoption of the report of the special nominating committee (Dr. W. W. Cort, Chairman, Drs. F. C. Bishopp, A. C. Chandler, E. C. Faust, J. F. Kessel, Benjamin Schwartz, H. W. Stunkard, D. H. Wenrich and J. E. Ackert, ex officio) which recommended that the present Editorial Committee of the JOURNAL OF PARASITOLOGY, Dr. N. R. Stoll, Chairman, Drs. W. H. Taliaferro and W. A. Riley, be elected for an additional term of one year beginning January 1, 1943.

(3) Passage of a unanimous vote of thanks to Dr. N. R. Stoll and his collaborators, Dr. and Mrs. M. S. Ferguson and Dr. George L. Graham, Dr. P. D. Harwood and Dr. A. C. Jerstad, for their painstaking work in preparing and publishing the Index for the first twenty-five volumes of the JOURNAL OF PARASITOLOGY.

(4) Election of Dr. Juan Bacigalupo, Buenos Aires, Argentina, as a Foreign Honorary Member of the Society.

Upon motion the report was accepted and placed on file.

2. The report of the Treasurer was presented by Dr. L. E. Rozeboom. The year's business was summarized as follows:

Credits			
Received for 1941	$3963.85		
Anticipated for 1941	93.60		
		$4057.45	
Debits			
Cost of Journal	$3300.54		
Chm. Ed. Comm.	$ 90.51		
Secretary	187.39		
Treasurer	390.39		
Miscellaneous ...	10.00		
	678.42		
		3978.96	
Balance from regular business			$78.49

In the estimate of receipts and expenditures for next year it was pointed out (1) that 55 non-member subscribers in enemy and occupied territory would be lost from the subscription list for the JOURNAL; (2) that the costs per page of publishing the JOURNAL would increase approximately 10 per cent; (3) that Council, in order to avoid a deficit, had voted to reduce the estimated number of pages to be published in the JOURNAL during 1942 from 600 to 540 pages. Upon motion, the report, certified as correct by the Auditing Committee, was accepted and placed on file.

II. REPORTS OF COMMITTEES

1. The report of the status of the Secretarial Fund prepared by Dr. N. R. Stoll, Custodian, was read by Dr. L. E. Rozeboom. Upon motion, the report, certified as correct by the Auditing Committee, was accepted and placed on file.

2. The report of the Editorial Committee prepared by Dr. N. R. Stoll, Chairman, was presented by Dr. H. W. Stunkard. Attention was called to the publication of the Index for Volumes 1–25 of the JOURNAL in August, 1941, and all mem-

bers were urged to take the responsibility of seeing to it that their college and departmental libraries secure copies as soon as possible. The sale in 1942 of at least 180 copies at $2.00 apiece is needed. The report noted that for regular articles the interval between "received for publication" and "date published" increased from an average of 6.5 months in 1940 to 10.7 months in 1941. Seventy-one articles and research notes are on hand (accepted or under consideration) and present acceptances are for the October number. It was also noted that beginning with the February, 1942, issue, reprints of articles will cost an estimated 10 per cent more than the present rates. Upon motion, the report was accepted with a vote of thanks to the Editorial Committee.

3. The report of the Committee for Cooperation with Biological Abstracts was given by Dr. George L. Graham, Chairman. A survey in April, 1941, showed that only 48 members of the Society were subscribers to one or more sections of Biological Abstracts. It was pointed out that the former practice of giving complete taxonomic citations for parasitological articles has been restored, and that abstracts are now appearing more promptly than ever before. Dr. George W. Hunter, III, suggested that abstracts covering medical entomology might be included in Section C without added cost to subscribers. Upon motion, a resolution was voted that the Society go on record as favoring the inclusion of abstracts of articles on medical entomology in Section C of Biological Abstracts.

4. A report of the Committee on the Terminology of Bird Malaria (copy appended) was explained briefly by Dr. R. D. Manwell. Upon motion, the report was accepted for publication in the JOURNAL.

III. Reports of Representatives of the Society

1. As representative on the Council of the American Association for the Advancement of Science, Dr. W. A. Riley gave a brief report of the Council meeting on the preceding day. New York City will be the meeting place of the Association in December, 1942.

IV. New Business

The following amendment of the Constitution proposed at the sixteenth annual meeting in Philadelphia was adopted by unanimous vote: "The Council shall consist of the President, the Vice-president, the Secretary, the Treasurer, *the Chairman of the Editorial Committee,* and eight members elected by ballot from the Society at large." (The phrase italicized represents the change adopted.)

Following the recommendation of the Council it was voted that Dr. George R. LaRue be authorized to organize in the name of the Society a program of parasitology at the summer meeting of the American Association for the Advancement of Science at Ann Arbor, Michigan, June 22–26, 1942, provided that no expense to the Society be incurred.

Dr. T. C. Nelson suggested that as a means of raising money for the Society it might be possible for members to donate extra research slides and material for sale to those wanting to purchase parasitological specimens. The proposal was discussed briefly but no formal action was taken.

The Secretary presented the Council nominations for officers of the Society for 1942, as follows: *President,* Henry E. Meleney; *Vice-president,* Rudolph W. Glaser; *Secretary,* for two years, Oliver R. McCoy; *Members of the Council,* for four years (to succeed E. C. Faust and H. J. Van Cleave), Raymond M. Cable and Willard H. Wright, for three years (to fill the unexpired term of N. R. Stoll), Gilbert F. Otto; *Members of the Editorial Board,* for four years (to succeed W. W. Cort, Benjamin Schwartz and D. H. Wenrich), W. W. Cort, Benjamin Schwartz and Harold Kirby, Jr.; *Representatives on the Council of the American Association for the Advancement of Science,* Asa C. Chandler and Harley J. Van Cleave; *Representatives on the Council of the Union of American Biological Societies,* George L. Graham and Arthur C. Walton. There were no further nominations and upon motion, duly

seconded, the Secretary was instructed to cast one ballot for the nominations as presented.

Upon motion, a vote of thanks was extended to Dr. E. W. Laake, local representative of the Society in Dallas, for his services in making arrangements for the luncheon and the demonstration program.

There being no further business, it was voted at 2:10 PM to adjourn until the next annual meeting in New York City in December, 1942.

Respectfully submitted,
O. R. McCoy, *Secretary*

CHANGES IN MEMBERSHIP OF THE AMERICAN SOCIETY OF PARASITOLOGISTS SINCE JUNE, 1941

New Members Elected

Louis J. Adolphsen	Harold Elishewitz	George Piternick
Robert M. Allen	William G. French	Irving Rappaport
G. F. Auguston	Jacob Frenkel	Nathan W. Riser
John L. Avery	Piero Gallo	Maurice M. Rothman
Gregory Bard	Frans G. Goble	Albert J. Samorodin
Aileen E. Bonestell	Theodore S. Hauschka	Albert J. Singer
William F. Cantrell	Philip A. Hawkins	Enrique G. Vogelsang
José C. M. Carvalho	Leon Jacobs	Joseph J. Volk
Frank H. Connell	John C. Johnson	Robert E. Ware
L. Reid Davis	Edward K. Markell	Albert J. Weathersbee
Willis H. Doetschman	Harry Most	J. Dan Webster
Wilbur G. Downs	Melvin Oosting	
Hilton H. Earle	Felise Pifano	

Resignations

Winterton C. Curtis	Rustum Maluf	Richard P. Strong
Anne Hager	George C. Shattuck	Zeferino Vaz

Deaths

Donald Cameron	Matthew E. Carroll, Jr.	Robert Hegner

SUPPLEMENT TO THE REPORT OF THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

Report of the Committee on Terminology of Strains of Avian Malaria

Proposals for a Uniform Terminology of Strains of Avian Malaria

A recognition of the growing need for some degree of standardization of the terminology of strains of avian malaria led to the appointment by President Wenrich in November, 1940, of a committee to consider this problem.

The need for some attempt at uniformity in terminology has become greater with: (1) the isolation of many strains of avian *Plasmodium* from wild birds, (2) the prevalent practice of the free exchange of such strains among laboratories and investigators, and (3) the discovery that the strains may change in one or more characteristics during laboratory study. Already there is some discrepancy in

designations given in publications to some of the strains by different investigators. It is the belief of the committee that prompt action on the part of malariologists in adopting a satisfactory terminology will avoid much needless confusion in the future.

The problems arising from the use of many strains of avian malaria and from their ability to change in character are greatly different from problems of zoological nomenclature. The former are more like those faced by bacteriologists in designating type cultures and their variants. For this reason the committee has decided that they should avoid as much as possible questions involving zoological nomenclature.

After studying a number of plans the committee has chosen the one which seemed to have the most to offer in convenience and accuracy. It is as follows:

1. Designations will consist of combinations of numerals and letters, beginning with a numeral.

2. The first portion of the designation, consisting of one or more numerals, corresponds to the species of *Plasmodium*. The species for which there are now strains in use will be designated approximately chronologically, i.e., *P. relictum* will be 1, *P. vaughani* will be 2, etc. The committee has no authority to change the specific identification given a strain by the investigator. Likewise, strain designations will have no weight in matters of priority of specific names. The committee will follow the desires of the investigator in manners of zoological classification. One numeral (50) will be reserved for strains not yet identified or described. The designations will be changed when the necessary information becomes available.

3. The letter, or letters, following the first numeral will indicate the original strain. Ordinarily this will apply to strains originating from natural infections of birds or the transmitting hosts. The letters which are already widely used will be preserved wherever possible, e.g., H, which has been used for Hartman's strain of *P. cathemerium*, will be retained. The letters O and I will not be employed because of the chance of confusion with numerals. When more than one letter is used in a designation there is no implied relationship to strains bearing one of these letters singly, e.g., HA is not necessarily related to H.

4. The numerals following the letters of the original strain designation (i.e., the second set of numerals) indicate sub-strains derived from originally isolated strains; thus the gametocyteless strain of *P. cathemerium* discovered by Huff and Gambrell becomes 3D1. It is recommended that sub-strains be designated only (1) when they are actually observed to differ from their parent strains, or (2) when some treatment is given the strain which is likely to result in change. Examples of the second type are the isolation of a clon, radical treatment by physical or chemical means, or passage through different hosts.

TABLE 1.—*Species designations*

Species numeral	Species name	Author	Date
1	*relictum*	Grassi and Filetti	1891
2	*vaughani*	Novy and MacNeal	1908
3	*cathemerium*	Hartman	1927
4	*rouxi*	Sergent, Sergent and Catanei	1928
5	*elongatum*	Huff	1930
6	*circumflexum*	Kikuth	1931
7	*polare*	Manwell	1934
8	*gallinaceum*	Brumpt	1935
9	*nucleophilum*	Manwell	1935
10	*hexamerium*	Huff	1935
11	*oti*	Wolfson	1936
12	*lophurae*	Coggeshall	1938
13	*durae*	Herman	1941
50	unidentified or undescribed species		

5. In order not to make the designation too cumbersome, further subdivisions of strains when they need to be made will be indicated by numerals separated from the rest of the designation by a hyphen; thus if a strain is derived from 3H2–1 or 3H2, which differs from its parent strain, it will be given a new terminal number,

TABLE 2.—*Designations of original strains*

Designation	Host source	Locality	Isolated by	Date of isolation	Other names used	Reference
P. relictum						
1 G	?	Hamburg, Germany	*	*	German	
1 H	*Carpodacus mexicanus frontalis* (Mexican finch)	Mexico City	Hewitt	1939	Mexican	J. Parasitol. **25** Suppl: 16 (1939)
1 M	*Passer domesticus* (English sparrow)	Syracuse, N. Y.	Manwell	1938	Var. *matutinum*	Am. J. Trop. Med. **20**: 859 (1940)
1 P	*Columba livia* (Pigeon)	Peru, Nebr.	Coatney	1938	Pigeon	Am. J. Hyg. **27**: 380 (1938)
1 R	*Planesticus migratorius* (American robin)	Kansas, Ill.	Huff	1935	Var. *matutinum*	J. Parasitol. **23**: 400 (1937)
1 T	*Hylocichla mustelina* (Wood thrush)	Baltimore, Md.	Wolfson	1936	Matinal	Am. J. Hyg. **25**: 177 (1937)
1 V	*Passer domesticus* (English sparrow)	Hampton, Va.	Huff	1926	Virginian	Am. J. Hyg. **7**: 706 (1927)
1 W	*Passer domesticus* (English sparrow)	New York City	Whitmore	1913	Whitmore	Bull. Johns Hopkins Hosp. **24**: 62 (1918)
P. vaughani						
2 R	*Planesticus migratorius* (American robin)	Syracuse, N. Y.	Manwell	1934		Am. J. Hyg. **21**: 180 (1935)
2 S	*Sturnus v. vulgaris* (Starling)	Baltimore, Md.	Hegner & Wolfson	1937		Am. J. Hyg. **28**: 437 (1938)
P. cathemerium						
3 A	*Quiscalus quiscala aeneus* (Bronzed grackle)	Paris, Ill.	Huff	1933	Grackle	J. Infect. Dis. **57**: 315 (1935)
3 B	*Agelaius phoeniceus* (Redwing)	Chino, Calif.	Herman			
3 D	Sparrow	Rome, Italy	Gingrich	1934	Hackett	Am. J. Hyg. **19**: 123 (1934)
3 H	*Passer domesticus* (English sparrow)	Baltimore, Md.	Hartman	1924	Hartman	Arch. Protistenk. **60**: 1 (1927)
3 M	*Pica pica hudsonia* (Magpie)	Nebraska	Coatney & Roudabush	1937	Magpie	Am. Midland Naturalist **18**: 1005 (1937)
3 Q	*Cacicus persicus* (Cacique)	South American bird in Chicago zoo	Huff	1941		
3 S	*Zonotrichia l. leucophrys* (White crowned sparrow)	Chino, Calif.	Herman	?		
3 T	*Hylocichla mustelina* (Wood thrush)	Baltimore, Md.	Wolfson	1936	Woodthrush or Wolfson	Am. J. Hyg. **31C**: 26 (1940)
P. rouxi						
4 A	Algerian sparrow	Algeria	Sergent, Sergent and Catanei	1928	"X"	Compt. Rend. Acad. Sc. **186**: 809 (1928)
P. elongatum						
5 A	*Passer domesticus* (English sparrow)	Kansas, Ill.	Huff	1932	Taber sparrow	J. Infect. Dis. **57**: 315 (1935)
5 B	*Quiscalus quiscala aeneus* (Bronzed grackle)	Kansas, Ill.	Huff	1933	Grackle	J. Infect. Dis. **57**: 315 (1935)

* Obtained by Gingrich in 1931 from Inst. f. Schiffs. u. Tropenhyg.

TABLE 2.—(*Continued*)

Designation	Host source	Locality	Isolated by	Date of isolation	Other names used	Reference
P. circumflexum						
6 A	*Melospiza m. melodia* (Song sparrow)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Trop. Med. **19**: 279 (1939)
6 B	*Planesticus migratorius* (American robin)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Hyg. **30**: 115 (1939)
6 C	*Zonotrichia albicollis* (White throated sparrow)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Trop. Med. **19**: 279 (1939)
6 D	*Zonotrichia albicollis* (White throated sparrow)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Hyg. **30**: 115 (1939)
6 E	*Agelaius phoeniceus* (Redwing)	Cape Cod, Mass.	Herman	1936		Am. J. Trop. Med. **19**: 279 (1939)
6 G	*Turdus pilaris* ("Thrush")	Germany	Kikuth	1931		Centr. Bakt. **121**: 401 (1931)
P. polare						
7 A	*Petrochelidon l. lunifrons* (Cliff swallow)	Westport, N. Y.	Manwell	1934		J. Parasitol. **22**: 412 (1936)
P. gallinaceum						
8 A	*Gallus domesticus* (Domestic chicken)	Ceylon	Brumpt	1935		Compt. Rend. Acad. Sc. **200**: 783 (1935)
P. nucleophilum						
9 A	*Dumatella carolinensis* (Catbird)	Syracuse, N. Y.	Manwell	1934		Am. J. Trop. Med. **15**: 265 (1935)
P. hexamerium						
10 A	*Melospiza m. melodia* (Song sparrow)	Syracuse, N. Y.	Manwell	1935		Am. J. Hyg. **27**: 196 (1938)
10 B	*Passerculus sandwichensis savanna* (Savannah sparrow)	Syracuse, N. Y.	Manwell	1941		
10 C	*Geothlypis t. trichas* (Maryland yellowthroat)	Syracuse, N. Y.	Manwell	1941		
10 J	*Sialia s. sialis* (Bluebird)	Kansas, Ill.	Huff	1934		Am. J. Hyg. **22**: 274 (1935)
P. oti						
11 A	*Asio otus naevius* (E. screech owl)	Glen Rock, Penn.	Wolfson	1936		Am. J. Hyg. **24**: 94 (1936)
P. lophurae						
12 A	*Lophura i. igniti* (Fire-backed pheasant)	Borneo (N. Y. zoo)	Coggeshall	1938	Strain 1	Am. J. Hyg. **27**: 615 (1938)
12 B	*Lophura i. igniti* (Fire-backed pheasant)	Borneo (N. Y. zoo)	Coggeshall	1938	Strain 2	Am. J. Hyg. **27**: 615 (1938)
P. durae						
13 A	*Meleagris gallopavo* (Domestic turkey)	Kenya Colony, E. Africa	Herman	1941		Am. J. Hyg. **34C**: 22 (1941)
Undescribed						
50 A	*Pediocetes phasianellus campestris* (Sharp-tailed grouse)	Lower Souris Refuge, N. D.	Wetmore	1939	*"P. pedioecetae"*	J. Wildlife Management **3**: 361 (1939)

e.g., 3H2–23 or whatever numeral is next available. In other words, the terminal portion of the designation (the portion following the hyphen) will not reflect obviously the genealogy of the strain. The genealogy will need to be sought from published accounts or from the committee.

The first series of designations are in Tables 1–3. The committee hopes to keep the list up to date by additions at such intervals as appear to be required.

Since the value of this system of terminology depends in large degree upon the cooperation of all malariologists working on avian malaria, the committee hopes that henceforth investigators when publishing the results of research will refer to

TABLE 3.—*Sub-strains of* P. cathemerium

Designation	Names in current usage	Derivation	Reference
3H1	Clon or "N" strain	Through single cell isolation by Stauber, at University of Chicago, 1933	J. Parasitol. **25**: 95 (1939)
3H2	Mosquito passage	Obtained from University of Chicago in March, 1938, since transferred exclusively by mosquito bite by Beckman, Marquette Medical School	Am. J. Trop. Med. **21**: 795 (1941)
3H3	Hartman-Hewitt; Phenylhydrazine	Virulence seemingly modified through treatment with phenylhydrazine by Hewitt at Johns Hopkins University	Am. J. Hyg. **29C**: 135 (1939)
3H1–1	"U"	Stauber's clon passed through 24 blood passages, then transmitted by *Culex pipiens* by Huff at University of Chicago, Oct. 1937	J. Infect. Dis. **68**: 184 (1941)
3H1–2	"P"	Stauber's clon passed through 30 blood passages, then transmitted by *Culex pipiens* by Huff at University of Chicago, Jan. 1939. Ability to produce normal numbers of gametocytes restored	J. Infect. Dis. **68**: 184 (1941)
3D1	Atypical; "F"; agametocyte strain	Gametocytes lost during rapid blood passage, Sept. 1932. Huff and Gambrell, University of Chicago. Periodicity disturbed; merozoite number decreased	Am. J. Hyg. **19**: 404 (1934)
3M1	None	Derived from small numbers of parasites in attempt to isolate clons. Less pathogenic for canaries than parent strain	None
3M2	None	Derived from small numbers of parasites in attempt to isolate clons. Less pathogenic for canaries than parent strain.	None

the strains used by the designations given by the committee. It is further urged that before publishing results of work on new strains or sub-strains investigators secure designations from the committee for such strains and incorporate them in their publications. It should be emphasized that the species name should be given in addition to the strain designation, e.g., "strain 3D1 of *P. cathemerium*."

COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA

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